UNIVERSIDAD AUTÓNOMA DE MADRID

FACULTAD DE CIENCIAS

Departamento de Química-Física Aplicada

Sección Departamental de Ciencias de la Alimentación

LACTATO DE ETILO COMO NUEVO DISOLVENTE VERDE PARA LA EXTRACCIÓN DE CAFEÍNA DE MATRICES VEGETALES

THE NEW GREEN SOLVENT ETHYL LACTATE FOR THE EXTRACTION OF CAFFEINE FROM VEGETABLE MATRICES

David Villanueva Bermejo

Tesis Doctoral

Madrid 2015

Instituto de Investigación en Ciencias de la Alimentación (CIAL, UAM-CSIC)

Departamento de Producción y Caracterización de Nuevos Alimentos

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Memoria presentada por:

David Villanueva Bermejo

Para optar al grado de

Doctor en Biología y Ciencias de la Alimentación

Con mención de Doctorado Internacional

Trabajo realizado bajo la dirección de:

Dra. Tiziana Fornari Reale

Dra. Elena Ibáñez Ezequiel

Instituto de Investigación en Ciencias de la Alimentación

DÑA. TIZIANA FORNARI REALE, PROFESORA TITULAR DE LA UNIVERSIDAD AUTÓNOMA DE MADRID Y DÑA. ELENA IBÁÑEZ EZEQUIEL, PROFESORA DE INVESTIGACIÓN DEL CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS.

INFORMAN,

Que el presente trabajo titulado **"Lactato de etilo como nuevo disolvente verde para la extracción de cafeína de matrices vegetales (The new green solvent ethyl lactate for the extraction of caffeine from vegetable matrices")**, y que constituye la memoria que presenta D. David Villanueva Bermejo para optar al grado de Doctor en Biología y Ciencias de la Alimentación, ha sido realizado bajo su dirección en la Universidad Autónoma de Madrid y en el Instituto de Investigación en Ciencias de la Alimentación (CIAL).

Y para que conste firman el presente informe en Madrid a 4 de septiembre de 2015.

Fdo. Tiziana Fornari Reale

Fdo. Elena Ibáñez Ezequiel

A mi familia y a Nerea.

Porque sois lo más importante

AGRADECIMIENTOS

Me gustaría comenzar dando las gracias a las principales responsables de que en este momento me encuentre escribiendo estas líneas. Gracias a las Dras. Tiziana Fornari y Elena Ibáñez, no solo por todo el aprendizaje científico que he adquirido trabajando con ellas y que queda reflejado en esta tesis, sino también, y muy especialmente, por ofrecerme todo aquello que no se muestra en ningún libro de ciencia, pero que para mí siempre ha sido igual o más importante, como el compromiso, la dedicación, la paciencia, la amabilidad y la cercanía. Siempre que lo he necesitado, habéis buscado tiempo para ayudarme inmediatamente, sin importar la cantidad de trabajo que teníais en ese momento ni el momento del día. Por todo ello, gracias de corazón.

Agradecer a la Sección Departamental de Ciencias de la Alimentación y en especial, al Dr. Guillermo Reglero Rada, el haberme brindado la oportunidad de iniciar mi carrera investigadora en el Grupo de Investigación de Ingredientes Alimentarios Funcionales de la Universidad Autónoma de Madrid,

Igualmente, agradecer a la directora del CIAL, la Dra. Mª Victoria Moreno, su amabilidad y profesionalidad, así como a todo el personal del CIAL por su dedicación y la gran labor que desempeñan diariamente.

Agradecer también al CSIC la concesión de la beca pre-doctoral del programa de Junta de Ampliación de Estudios (JAE), que me ha permitido realizar esta tesis, y a la Universidad Autónoma de Madrid la beca concedida para la realización de una estancia investigadora en el extranjero.

Gracias al Dr. José A. Mendiola y a la Dra. Mónica Rodríguez. Gracias José por tu paciencia y por buscar siempre un momento para ayudarme y gracias Mónica por ofrecerme tu ayuda siempre que lo he necesitado y por responder a tantas y tantas preguntas siempre con una sonrisa.

Gracias a la Dra. Charlotta Turner y a todos los miembros del Green Technology Group de la Universidad de Lund (Suecia) por acogerme de ese modo y por la gran experiencia vivida durante mi estancia pre-doctoral y, en especial, gracias a las Dras. Irene Rodríguez y Merichel Plaza por todo lo que hicieron antes y durante mi estancia. Gracias Irene por todo lo que me has enseñado y gracias a ambas por todo vuestro esfuerzo y ayuda. Hicisteis que me sintiera como en casa.

Gracias a mis compañeros, porque por su "culpa" esta ha sido una de las mejores épocas de mi vida. Gracias a Erika, Gonzalo y Alexis, mis compañeros de laboratorio, por todo lo que he aprendido y por todo lo que he reído y disfrutado con ellos. Gracias a Bienve, Andrea, Gloria, Javi, Maika, Inés, Alba, Ana, Marta, Peppe, Fabián, Ana María, Alicia, Marisol, Tanize, por los buenos momentos que hemos pasado juntos. Y…cómo no…gracias a Lidia y a Juanan, con los que he tenido la grandísima suerte de vivir toda esta etapa, con los que he compartido los mejores momentos y deseo seguir compartiéndolos. Gracias por ser como sois, por escucharme y ofrecerme siempre vuestra ayuda y por permitirme disfrutar a vuestro lado (Juanan, qué más te podría decir…otra etapa que terminamos juntos. Ahora, juntos a por la siguiente).

Y por supuesto, gracias a mi familia, las personas que siempre están a mi lado, en los buenos y malos momentos, porque ellos son lo más importante.

Por último, me gustaría dedicar estas últimas líneas a una persona que, de no haberla conocido, no habría podido disfrutar este momento. Gracias Nerea por todo tu apoyo, por tu paciencia y por comprenderme mejor que nadie. Solo espero poder devolver algún día la mitad de lo que me has dado, porque devolverte todo es imposible.

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RESUMEN

RESUMEN

Los crecientes problemas medioambientales y las mayores evidencias científicas acerca de los efectos adversos sobre la salud de ciertos compuestos químicos frecuentemente utilizados en la producción industrial, ha traído consigo la demanda de procesos medioambientalmente más sostenibles y seguros. De forma paralela, el creciente interés social por la alimentación y su relación con la salud ha impulsado el desarrollo de los llamados alimentos funcionales, es decir, alimentos cuyo consumo afecta beneficiosamente a una o más funciones del organismo, más allá de los efectos nutritivos propios del alimento, de modo que contribuyen a mejorar el estado de salud y/o reducir el riesgo a padecer alguna enfermedad.

Un procedimiento frecuente para desarrollar y producir un alimento funcional consiste en la extracción de matrices vegetales para obtener un ingrediente con actividad biológica, el cual se utilizará para diseñar el alimento funcional. Un ejemplo es la extracción de fitosteroles, compuestos que contribuyen a reducir el nivel de colesterol en sangre, y que se añaden como ingrediente funcional en la elaboración de margarinas. En otros casos, la extracción se lleva a cabo para eliminar ciertas sustancias del propio alimento, que pueden presentar un efecto adverso o indeseable sobre la salud. En ambos casos, el desarrollo de procesos eficientes, que utilicen tecnologías medioambientalmente limpias y disolventes ecológicos, aceptados en la industria alimentaria, es un factor esencial a tener en cuenta.

El café y el té son dos productos alimentarios que concentran un gran interés comercial, ya que las bebidas elaboradas a partir de ellos son las más consumidas a nivel mundial. La composición química de estos productos ha sido muy estudiada y en ella se han descrito ciertos compuestos con actividad biológica, entre los que se encuentra la cafeína. Esta sustancia es el principal alcaloide consumido en el mundo y su actividad como estimulante del sistema nervioso central es bien conocida. Algunos de los efectos negativos asociados a su consumo excesivo son el aumento en la presión arterial, insomnio, ansiedad o taquicardia. Debido a estos efectos perjudiciales, así como a la mayor prevalencia de ciertas enfermedades crónicas, como la hipertensión, extendida fundamentalmente en los países occidentales, se ha generado en los últimos años un gran aumento en la demanda de productos descafeinados. En la producción de café y té descafeinado el objetivo no solo es obtener un producto prácticamente exento

de cafeína, sino también obtener un producto de buena calidad, minimizando la pérdida de sustancias que le confieren aroma y sabor a la bebida, así como recuperar y purificar la cafeína extraída, para que pueda ser utilizada en la producción de ciertos alimentos (por ejemplo, refrescos) y en la industria farmacéutica.

Actualmente, a nivel comercial, el proceso de descafeinado utilizando dióxido de carbono supercrítico está muy extendido. Este proceso constituye la primera aplicación de la tecnología supercrítica a nivel industrial en el campo de la producción de alimentos. En todos los procedimientos de descafeinado supercrítico patentados es común utilizar agua como cosolvente, ya sea simplemente realizando una hidratación previa del material vegetal, con el objetivo de facilitar la disponibilidad y difusión de la cafeína hacia el fluido supercrítico, o además, saturando de agua el fluido supercrítico al hacerlo pasar a través de un reservorio que contenga agua. De esta manera, el dióxido de carbono supercrítico ha demostrado ser muy selectivo y eficaz en la extracción de cafeína.

No obstante, el primer proceso industrial de descafeinado se llevó a cabo con disolventes líquidos y actualmente este procedimiento continúa utilizándose, empleando disolventes clorados como el cloroformo o el cloruro de metileno, los cuales han demostrado ser muy selectivos y eficientes en el descafeinado de café y té. No obstante, su uso ha ido disminuyendo debido a su toxicidad y a los problemas medioambientales que generan. Actualmente, el agua y el acetato de etilo, un disolvente orgánico con una baja toxicidad y más ecológico que los disolventes clorados, son los principales disolventes líquidos utilizados en el descafeinado comercial de café y té. Sin embargo, estos disolventes no presentan el mismo grado de selectividad que los disolventes clorados, especialmente el agua, por lo que durante el proceso se produce la extracción de otros compuestos diferentes a la cafeína, que podrían afectar a la calidad sensorial del producto descafeinado. Otra desventaja de estos procedimientos de extracción sólido-líquido son los largos tiempos de extracción y los elevados volúmenes de disolvente empleados, que posteriormente deben ser tratados y/o eliminados.

Por ello, la búsqueda de nuevos disolventes líquidos de extracción, ecológicos y selectivos para la extracción de cafeína procedente de estas matrices vegetales, ya sean solos o combinados con dióxido de carbono supercrítico, constituye un interesante campo de estudio.

El lactato de etilo es un disolvente agroquímico, designado GRAS (Generally Recognized as Safe) y que, debido a su baja toxicidad, ha sido aprobado por la FDA (Food and Drug Administration) y por la EFSA (European Food Safety Authority) como aditivo alimentario y farmacéutico. El lactato de etilo es un disolvente utilizado en diversos sectores industriales debido a sus interesantes propiedades, como por ejemplo, no ser corrosivo, no ser carcinogénico ni teratogénico, ser biodegradable y no ser nocivo para la capa de ozono, así como presentar un elevado poder solvente, abarcando un amplio rango de polaridades. No obstante, el lactato de etilo ha sido poco estudiado como disolvente de extracción de compuestos alimentarios y en este caso, los trabajos se han orientado principalmente a la extracción y fraccionamiento de compuestos de naturaleza lipídica (escualeno y α-tocoferol), recuperación de esclareol procedente de Salvia, extracción de carotenoides, en concreto, licopeno, luteína, β-caroteno, y astaxantina, de distintas matrices vegetales, y extracción de ácido γ-linolénico procedente del microalga Spirulina. Igualmente se ha utilizado para la extracción de distintos compuestos fenólicos procedentes de retama negra. En esta tesis, se llevó a cabo un trabajo detallado de revisión bibliográfica acerca de las propiedades y aplicaciones del lactato de etilo, que dio lugar a la publicación titulada "*Ethyl lactate: a biorenewable agrochemical solvent for food technology*" del libro Handbook of Solvents, Volume 2, Use, Health, and Environment (ISBN 978-1-895198-65-2).

En base a lo expuesto anteriormente, en la presente memoria se llevaron a cabo varios estudios de solubilidad y selectividad con el objetivo de evaluar el uso del lactato de etilo como disolvente de extracción de cafeína de matrices vegetales, en concreto, granos de café verde y hojas de té verde.

Por un lado, se midió la solubilidad de varios compuestos alimentarios en lactato de etilo (timol, ácido ferúlico, ácido vainíllico, ácido cafeico y cafeína) en un rango de temperatura entre 25 ºC y 70 ºC. En relación a la cafeína, se observó que su solubilidad en lactato de etilo fue más alta que la solubilidad en otros disolventes orgánicos estudiados, como acetona, metanol, etanol o acetato de etilo, y similar a la solubilidad en agua. Asimismo, se observó un aumento considerable de la solubilidad de cafeína en mezclas lactato de etilo + agua, respecto a la correspondiente solubilidad en los disolventes puros, determinándose así un efecto cosolvente entre el agua y el lactato de etilo. Estos estudios dieron lugar al trabajo titulado "*Solubility of high-value compounds*

in ethyl lactate: measurements and modeling" (Journal of Chemical Thermodynamics, 48 (2012) 93-100).

Igualmente, se definió el diagrama de fases líquido-vapor del sistema binario lactato de etilo + dióxido de carbono, estableciendo así las condiciones de temperatura y presión que permiten la formación de una fase supercrítica homogénea, información básica para el desarrollo de procesos supercríticos que incluyan el uso de dióxido de carbono y lactato de etilo. Los resultados se publicaron en el trabajo titulado "*Solubility of CO_2 in ethyl lactate and modeling of the phase behavior of the CO_2 + ethyl lactate mixture*" (Journal of Chemical and Engineering Data, 58 (2013) 301-306).

En base a estos resultados y, teniendo en cuenta la importancia comercial que tienen los productos de café y té descafeinados, se llevó a cabo el estudio experimental del lactato de etilo como disolvente de extracción de cafeína procedente de estas matrices vegetales.

En las extracciones de cafeína de granos de café verde y hojas de té verde, además de lactato de etilo, se estudiaron otros disolventes líquidos, en concreto, etanol, acetato de etilo y agua, con el objetivo de establecer una comparación apropiada. En este estudio se empleó la extracción con líquidos presurizados (PLE) por ser una técnica de extracción más eficiente que la extracción sólido-líquido convencional, ya que ésta emplea tiempos de extracción más cortos y un menor volumen de disolventes.

Los resultados obtenidos en el presente trabajo demuestran que el lactato de etilo y sus mezclas con agua son buenos disolventes de extracción de cafeína y podrían utilizarse para la producción de café y té descafeinados, constituyendo una alternativa ecológica a los disolventes clorados, y mejorando la selectividad respecto a otros disolventes ecológicos actualmente utilizados, como el acetato de etilo y el agua. Estos trabajos dieron lugar a los artículos "*Extraction of caffeine from natural matter using a bio-renewable agrochemical solvent*" (Food and Bioproducts Processing, 91 (2013) 303-309) y "*Pressurized liquid extraction of caffeine and catechins from green tea leaves using ethyl lactate, water and ethyl lactate + water mixtures*" (Food and Bioproducts Processing, 96 (2015) 106-112).

Asimismo, con el objetivo de estudiar el efecto del lactato de etilo como cosolvente en procesos de extracción con dióxido de carbono supercrítico, se llevó a cabo la

extracción de hojas de té verde, para evaluar la eficacia del lactato de etilo, etanol y acetato de etilo como cosolventes de extracción de cafeína. Nuevamente, de los tres cosolventes empleados, el lactato de etilo resultó ser el más eficaz, ya que con él se obtuvo la mayor recuperación de cafeína y los mayores coeficientes de transferencia de cafeína a la fase supercrítica. Estos resultados dieron lugar al trabajo titulado "*Effect of cosolvents (ethyl lactate, ethyl acetate and ethanol) on the supercritical CO_2 extraction of caffeine from green tea*" (The Journal of Supercritical Fluids, en prensa).

Por otro lado, en el descafeinado de hojas de té verde, además de la eliminación de cafeína, es importante minimizar la extracción de otros compuestos distintos a la cafeína, como por ejemplo las catequinas, las cuales contribuyen a las características sensoriales del producto final y cuyo consumo se ha asociado a una serie de propiedades beneficiosas para la salud. Sin embargo, durante el descafeinado es inevitable la pérdida de cierta cantidad de catequinas. En este sentido, se han llevado a cabo distintos estudios en la bibliografía con el objetivo de producir un extracto rico en catequinas y con bajo contenido de cafeína, que pueda ser utilizado como ingrediente funcional en la elaboración de bebidas embotelladas y productos de repostería, entre otros. Entre las técnicas utilizadas se encuentran la tecnología de membranas, el uso de adsorbentes o el fraccionamiento con disolventes orgánicos. Pese a ello, los resultados obtenidos no son muy satisfactorios, obteniéndose bajos rendimientos de producción y/o extractos con un contenido relativamente alto de cafeína o una baja relación catequinas / cafeína. En la presente tesis se desarrolló un proceso combinado de extracción de hojas de té verde con lactato de etilo presurizado, seguido de un fraccionamiento y precipitación utilizando dióxido de carbono supercrítico como gas antisolvente, lográndose un extracto con un 23 % de catequinas y menos de 1 % de cafeína (% m/m). Los resultados dieron lugar a la publicación "*High catechins/low caffeine powder from green tea leaves by pressurized liquid extraction and supercritical antisolvent precipitation*" (Separation and Purification Technology, 148 (2015) 49-56).

Por tanto, en esta tesis se demuestra que la aplicación de lactato de etilo en procesos de extracción y/o fraccionamiento de cafeína de matrices vegetales, para la producción de alimentos o ingredientes descafeinados, es un procedimiento potencialmente viable para la industria alimentaria, reemplazando con mayor selectividad y eficacia a otros disolventes verdes actualmente utilizados para este fin.

ABSTRACT

The growing environmental problems and the increasing scientific evidences related to the adverse health effects of certain chemical compounds frequently used in the industrial production have influenced in the demand of efficient production processes, more environmentally sustainable and safe. At the same time, the growing social interest towards food and its connection with health has motivated the development of the so-called functional foods, that is, foods whose consumption has a beneficial effect on one or more body functions beyond its nutritive effect, so that they contribute to improve the health status and/or to decrease the risk of developing a particular disease.

A common procedure to develop and produce a functional food consists in the extraction from vegetable matrices to obtain an ingredient with biological activity which will be used to design the functional food. An example is the extraction of phytosterols that contribute to decrease the blood cholesterol level and are added as functional ingredient in the production of margarines. In other cases, the extraction is carried out to remove from the food certain compounds that exhibit an adverse health effect. In both cases, the design of sustainable processes that use environmentally friendly technologies and green solvents, whose use is permitted in food industry, is a key aspect to take into account.

Coffee and tea are two foodstuffs which focus a great commercial attention since beverages elaborated from them are the most consumed worldwide. The chemical composition of these products has been thoroughly studied and several compounds with biological activity have been described, among them caffeine. This compound is the main worldwide consumed alkaloid and its activity as central nervous system stimulant is well known. Some of the negative health effects derived from its intake comprises an increase in blood pressure, insomnia, anxiety or tachycardia. Owing to these detrimental effects as well as the greater prevalence of certain chronic diseases, such as hypertension that is mainly happening in occidental countries, a large increase in the decaffeinated product market has been observed in recent years. In the production of decaffeinated coffee and tea not only is interesting to obtain a final product practically free of caffeine, but also to obtain a high quality product, minimizing the loss of scent and flavor-related compounds as well as recovering and purifying the removed caffeine

for further use as a food ingredient (for example, added to energy drinks) and in the pharmaceutical industry.

At present, the decaffeination process using supercritical carbon dioxide is very widespread commercially. This process constitutes the first application of the supercritical technology at industrial scale in the food production field. In all patented supercritical decaffeination processes is common to use water as cosolvent either by moistening the vegetable material before extraction to improve the availability and diffusion of caffeine toward the supercritical fluid or saturating the supercritical fluid with water forcing it to pass through a water reservoir. In this way, supercritical carbon dioxide has proved to be a very selective and efficient solvent for the extraction of caffeine.

Nevertheless, the first decaffeination industrial process was carried out using liquid solvents and this procedure is currently being used with chlorinated solvents such as chloroform or dichloromethane, which have proven to be quite selective and efficient for the decaffeination of coffee and tea. Despite this, the use of these solvents has decreased due to its high toxicity and their associated environmental problems. At present, water and ethyl acetate, which is an organic solvent with low toxicity and greener than chlorinated solvents, are the main commercially used liquid solvents for decaffeinating coffee and tea. However, these solvents do not show the same selectivity degree as chlorinated solvents, especially water, so non-caffeine compounds are co-extracted during the process that can affect the sensorial quality of the decaffeinated product. Another drawback of solid-liquid extraction procedures is the large extraction times and high solvent volumes required which should be treated and/or removed afterwards.

For this reason, the search of new green and selective extraction liquid solvents, to be used either in a pure form or added to supercritical carbon dioxide for the extraction of caffeine from vegetable matrices, constitutes an interesting field of study.

Ethyl lactate is an agrochemical solvent defined as GRAS (Generally Recognized as Safe) and due to its low toxicity it has been approved by FDA (Food and Drug Administration) and EFSA (European Food Safety Authority) as a pharmaceutical ingredient and food additive. Ethyl lactate is a solvent used in different industrial areas owing to their interesting properties, e.g. ethyl lactate is a non-corrosive, non-

carcinogenic, non-teratogenic, biodegradable and non-ozone depleting, as well as showing a great solvent power and reaching a wide polarity range. In spite of this, ethyl lactate has been poorly studied as an extraction solvent for food compounds and in this case, the studies have mainly been focused on the extraction and fractionation of lipid related compounds (squalene and α-tocopherol), the recovery of sclareol from Salvia, the extraction of carotenoids, in particular lycopene, lutein, β-carotene and astaxanthin from different vegetable matrices and the extraction of γ-linolenic acid from Spirulina microalgae. Additionally, ethyl lactate has been used for the extraction of different phenolic compounds from common broom. In the present dissertation, a detailed literature review in relation to the properties and applications of ethyl lactate was carried out and it led to the publication titled "*Ethyl lactate: a biorenewable agrochemical solvent for food technology*" published in the book Handbook of Solvents, Volume 2, Use, Health, and Environment (ISBN 978-1-895198-65-2).

Based on the foregoing, several solubility and selectivity studies were carried out in in the present report with the aim of evaluating the use of ethyl lactate as an extraction solvent of caffeine from vegetable matrices, specifically green coffee beans and green tea leaves.

On one hand, the solubility of some food compounds in ethyl lactate (thymol, ferulic acid, vanilic acid, cafeic acid and caffeine) was measured in a temperature range between 25 ºC and 70 ºC. Regarding caffeine, it was observed that its solubility in ethyl lactate was higher than that obtained with other organic solvents previously studied, such as acetone, methanol, ethanol or ethyl acetate, and similar to the solubility in water. Additionally, a substantial increase in the solubility of caffeine in (ethyl lactate + water) mixtures, with respect to the corresponding solubility in pure solvents was observed, so a co-solvent effect between water and ethyl lactate was determined. These studies gave rise to the publication titled "*Solubility of high-value compounds in ethyl lactate: measurements and modeling*" (Journal of Chemical Thermodynamics, 48 (2012) 93-100).

Likewise, the liquid-vapor phase diagram of the binary system (ethyl lactate + carbon dioxide) was defined, therefore establishing the temperature and pressure conditions allowing the formation of a homogeneous supercritical phase. This information is essential to develop supercritical processes where carbon dioxide and

ethyl lactate are involved. The results were reported in the work titled "*Solubility of CO_2 in ethyl lactate and modeling of the phase behavior of the CO_2 + ethyl lactate mixture*" (Journal of Chemical and Engineering Data, 58 (2013) 301-306).

Considering these results and taking into account the commercial significance of decaffeinated coffee and tea products, the experimental study of ethyl lactate as an extraction solvent of caffeine from these vegetable matrices was carried out.

Besides ethyl lactate, other liquid solvents specifically ethanol, ethyl acetate and water were studied in the extractions of caffeine from green coffee beans and green tea leaves in order to establish an appropriate comparison. The pressurized liquid extraction (PLE) was used in this study since it is a more efficient extraction technique than the traditional solid-liquid extraction due to the use of shorter extraction time and lesser solvent volumes.

The results obtained in the present work demonstrate that ethyl lactate and their mixtures with water are good solvents for caffeine extraction, and that they could be used to produce decaffeinated coffee and tea, being a green alternative to chlorinated solvents and improving the selectivity with respect to other green solvents, such as ethyl acetate and water which nowadays are used. These works led to the articles "*Extraction of caffeine from natural matter using a bio-renewable agrochemical solvent*" (Food and Bioproducts Processing, 91 (2013) 303-309) and "*Pressurized liquid extraction of caffeine and catechins from green tea leaves using ethyl lactate, water and ethyl lactate + water mixtures*" (Food and Bioproducts Processing, 96 (2015) 106-112).

In addition, with the aim of studying the effect of ethyl lactate as a cosolvent in the extraction process using supercritical carbon dioxide, the extraction was carried out on green tea leaves in order to evaluate the efficiency of ethyl lactate, ethanol and ethyl acetate as caffeine extraction cosolvents. Once again, ethyl lactate was the most efficient of the three cosolvents studied since its use provides with the highest recovery of caffeine and the largest caffeine transfer coefficient to the supercritical phase were attained. These results gave rise to the work titled "*Effect of cosolvents (ethyl lactate, ethyl acetate and ethanol) on the supercritical CO_2 extraction of caffeine from green tea*" (The Journal of Supercritical Fluids, in press).

On the other hand, besides the extraction of caffeine, it is important to minimize the extraction of other compounds during the decaffeination of green tea leaves, for example catechins, which contribute to the organoleptic properties of the final product and whose intake has been associated to a series of beneficial effects on health. Nevertheless, the loss of a certain amount of catechins is unavoidable during decaffeination. In this sense, different studies have been reported in the literature in which the objective was to extract a fraction enriched in catechins and with low caffeine content, to be used as a functional ingredient in the production of canned drinks and confectionery products, among others. Membrane technologies, the use of adsorbents or solvent partitioning have been employed for this purpose. Nonetheless, the results are unsatisfactory due to low production yields and/or extracts with relatively high caffeine content or low catechins / caffeine ratio. In the present PhD Dissertation, a combined process based on the extraction of green tea leaves using pressurized ethyl lactate, followed by a selective fractionation and precipitation procedure using the supercritical carbon dioxide as antisolvent fluid was developed. A precipitate with less than 1 % of caffeine and 23 % of catechins (% m/m) was obtained. The results were reported in the work "*High catechins/low caffeine powder from green tea leaves by pressurized liquid extraction and supercritical antisolvent precipitation*" (Separation and Purification Technology, 148 (2015) 49-56).

Therefore, in the present thesis, we have been able to demonstrate that the application of ethyl lactate in caffeine extraction and/or fractionation processes from vegetable matrices in order to produce decaffeinated foods or ingredients is a potentially viable procedure for food industry, replacing with more selectivity and efficiency other green solvents which are being used to this end.

1

INTRODUCCIÓN

1. INTRODUCCIÓN

1.1. QUÍMICA VERDE

A mediados del siglo XX eran considerables los problemas medioambientales originados como consecuencia de la intensificación de la producción de bienes. Por otro lado, a este problema se añadieron las distintas confirmaciones acerca de los efectos adversos sobre la salud de numerosos compuestos químicos utilizados frecuentemente en la industria. Estos problemas originaron el desarrollo de nuevas regulaciones acerca de la generación y eliminación de residuos por parte de la industria y la aparición del concepto de Química Verde.

Química Verde es un concepto relativamente nuevo, formulado por primera vez en Estados Unidos en 1991 y aplicable a todos los sectores industriales (Anastas y Eghbali, 2010). Desde su aparición, este concepto ha sido adoptado por numerosos gobiernos, llevándose a cabo cientos de programas e iniciativas por todo el mundo para promover los principios que sustenta.

La Química Verde consiste en el diseño, desarrollo y aplicación de productos químicos y procesos que reducen o eliminan el uso y generación de sustancias peligrosas, abarcando el término peligroso un sentido amplio, incluyendo consideraciones físicas (por ejemplo, flamabilidad), toxicológicas (por ejemplo, mutagenicidad), además de medioambientales (Anastas y Kirchhoff, 2002).

No existe un método sistemático y fiable para asegurar que un producto o proceso implementado sea ecológico, debido principalmente al enorme número de procesos y vías de síntesis química que se pueden dar y a que los conceptos de la Química Verde pueden ser aplicados en cualquier punto del ciclo de vida, desde el origen de la materia prima hasta más allá del final de la vida útil (Anastas y Kirchhoff, 2002). Así, sólo es posible verificar si un proceso y/o producto es más ecológico que otra alternativa. En este sentido, Anastas y Warner (Anastas y Warner, 1998) desarrollaron los Doce Principios de la Química Verde, una serie de recomendaciones y criterios sobre cómo diseñar un proceso y/o un producto de forma más ecológica. Estos principios son los siguientes:

1.- Prevención: es preferible prevenir la generación de un residuo que tratarlo o limpiarlo una vez formado.

2.- Economía atómica: los procesos de síntesis deberían diseñarse de manera que se maximice la incorporación de todos los materiales usados durante el proceso en el producto final, minimizándose así la cantidad de subproductos generados.

3.- Procesos de síntesis química más seguros: siempre que sea posible, los procesos de síntesis deberían diseñarse de forma que se utilicen y generen sustancias que posean una baja o nula toxicidad y no sean nocivos para el medioambiente.

4.- Generación de productos más seguros: los productos deberían diseñarse de forma que mantengan su funcionalidad y eficacia a la vez que se reduzca su toxicidad.

5.- Reducción del uso de sustancias auxiliares: el uso de sustancias auxiliares (como, por ejemplo, disolventes, agentes de separación, etc.) debería evitarse siempre que sea posible y, en caso de requerirse, ser lo más inocuos posible.

6.- Reducción del consumo energético: el impacto económico y medioambiental de los requerimientos energéticos en los procesos químicos debería determinarse y minimizarse. Siempre que sea posible, los procesos deberían llevarse a cabo a presión y temperatura ambiente.

7.- Utilización de materias primas renovables: siempre que sea técnicamente y económicamente posible se utilizarán materias primas renovables.

8.- Reducción de los derivados: siempre que sea posible, la derivatización se reducirá o evitará, ya que la formación de derivados requiere la utilización de reactivos y disolventes adicionales, por lo que pueden generarse más residuos.

9.- Catálisis: es preferible potenciar el uso de reactivos catalíticos, lo más selectivos posibles, frente al empleo de reactivos estequiométricos.

10.- Degradación de productos: los productos químicos deben diseñarse de forma que al final de su vida útil no persistan en el medio y se degraden originando productos inocuos.

11.- Análisis en tiempo real para la prevención de la contaminación: se desarrollarán metodologías analíticas que permitan monitorizar y controlar en tiempo real la formación de sustancias peligrosas.

12.- Prevención de los accidentes químicos: se seleccionarán las sustancias y sus formas de uso con el objetivo de minimizar el riesgo potencial de accidentes químicos, incluido fugas, explosiones e incendios.

En el caso concreto del sector alimentario, el desarrollo económico, cultural y científico que se ha producido en la sociedad y que ha servido de impulso en las dos últimas décadas para el desarrollo de los alimentos funcionales o alimentos de uso específico para la salud (Diplock y col., 1999), ha traído consigo la demanda de procesos de producción medioambientalmente más sostenibles y seguros.

Una de las estrategias para la elaboración de alimentos funcionales consiste en una etapa de extracción en la que se aísla el componente activo (generalmente de una fuente de origen vegetal) el cual posteriormente se añade en una cierta dosis a una matriz base (producto cárnico, lácteo, una bebida, etc.). La utilización de disolventes ecológicos, no tóxicos, y el uso de procesos menos contaminantes y más eficientes, es un aspecto esencial a tener en cuenta en el diseño y desarrollo de la etapa de extracción.

Las últimas tendencias se han centrado principalmente en el papel de los disolventes, tanto para minimizar su consumo, como para buscar alternativas a disolventes tradicionalmente utilizados en la industria. En este sentido, Chemat y col. (2012) definieron la "extracción verde" como aquella basada en el diseño y desarrollo de procesos de extracción energéticamente más eficientes, en los que se utilicen disolventes renovables y que garantice una alta calidad y seguridad del extracto o del producto obtenido. Para llevarlo a cabo, los autores definieron seis recomendaciones:

1.- Seleccionar y utilizar recursos vegetales renovables.
2.- Utilizar disolventes alternativos, principalmente agua o disolventes agroquímicos.
3.- Reducir el consumo de energía mediante su recuperación y el uso de tecnologías innovadoras.
4.- Generar co-productos en lugar de residuos, para así poder ser destinados a biorrefinerías.
5.- Reducir las operaciones unitarias y favorecer la seguridad, robustez y el control de los procesos.
6.- Producir extractos biodegradables libres de contaminantes.

Es por ello que el estudio de disolventes alternativos, que reemplacen a los derivados de petróleo, y el desarrollo de procesos que se lleven a cabo mediante técnicas novedosas de extracción se ha intensificado en las últimas décadas.

1.2. LACTATO DE ETILO

Muchos de los disolventes tradicionales, como son los disolventes halogenados y los derivados del petróleo, además de generar un gran impacto medioambiental, son tóxicos, inflamables y/o corrosivos. Una mayor conciencia social, así como el endurecimiento de la legislación en materia medioambiental que se ha producido a lo largo de los últimos años, ha promovido la intensificación en el estudio de nuevos disolventes, en un intento por sustituir a los disolventes tradicionales. En este sentido, los ésteres del ácido láctico se han presentado como candidatos a sustituir a muchos de ellos (Sheldon, 2005).

Uno de estos ésteres es el lactato de etilo (2-hidroxipropanoato de etilo), que proviene de la esterificación de ácido láctico con etanol (Figura 1.1), llevada a cabo en biorrefinerías a partir de subproductos agrícolas ricos en carbohidratos, como por ejemplo, residuos del maíz o residuos de la industria azucarera (Pereira y col., 2011).

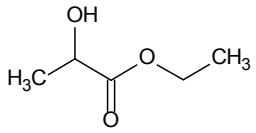


Figura 1.1. Estructura molecular del lactato de etilo

El lactato de etilo aparece de forma natural y en pequeñas cantidades en una amplia variedad de alimentos, como carne, algunas frutas, productos de soja y ciertas bebidas fermentadas, como vino y cerveza, formando parte del aroma de estos alimentos. Como disolvente agroquímico constituye una alternativa viable a los disolventes tradicionales debido a sus propiedades, tales como no ser corrosivo, no ser carcinogénico ni teratogénico, ser biodegradable y no ser nocivo para la capa de ozono. El lactato de etilo ha sido designado como GRAS (Generally Recognized as Safe) y debido a su baja toxicidad ha sido aprobado por la FDA (Food and Drug Administration) y por la EFSA (European Food Safety Authority) como aditivo alimentario y farmacéutico (Pereira y Rodrigues, 2014).

Respecto a sus propiedades físico-químicas, el lactato de etilo tiene un bajo punto de ebullición, una viscosidad no demasiado elevada, una densidad similar a la del agua, baja presión de vapor y una baja tensión superficial, así como un alto poder solvente, ya

que puede abarcar un amplio rango de polaridades (Pereira y Rodrigues, 2014). El lactato de etilo puede formar puentes de hidrógeno debido al hidrógeno del grupo hidroxilo y al oxígeno alcoxi y carbonilo que presenta en su estructura. Su constante dieléctrica de 15,7 a 25 ºC, hace que se le pueda considerar un disolvente moderadamente polar (Aparicio y Alcalde, 2009).

Debido a su carácter agroquímico y a las propiedades anteriormente mencionadas, se han estudiado y patentado muchos procesos con el objetivo de reducir su coste de producción y promocionar su uso como alternativa a los disolventes no ecológicos.

El lactato de etilo puede generarse principalmente a través de dos vías (Figura 1.2). Por un lado, a través de la esterificación de ácido láctico con etanol, originándose agua como subproducto de la reacción. Por otro lado, el lactato de etilo puede producirse esterificando etanol con lactato amónico. La fermentación en presencia de amonio para producir lactato amónico, y su posterior reacción con etanol, origina lactato de etilo y amoniaco como productos (Kwak y col., 2012).

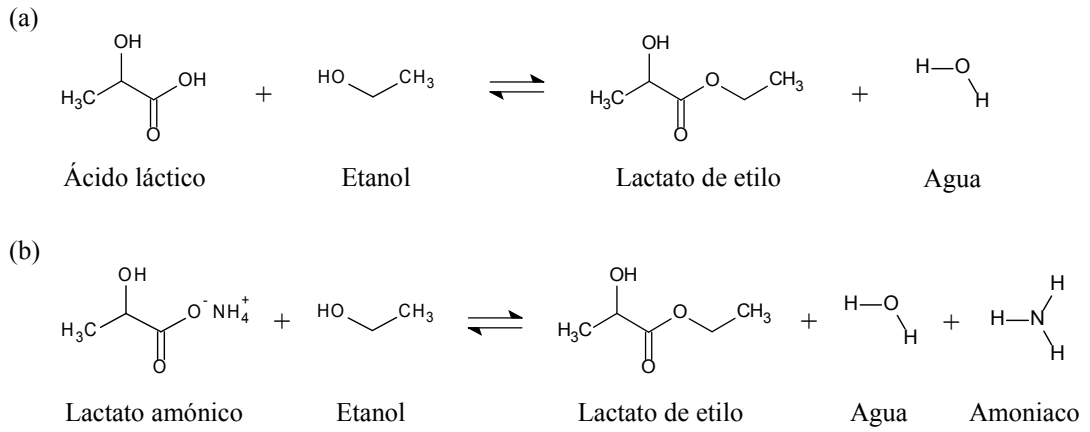


Figura 1.2. Métodos de producción del lactato de etilo: (a) esterificación de ácido láctico con etanol, (b) esterificación del lactato amónico con etanol.

El lactato de etilo también puede obtenerse utilizando como materia prima lactida (un dímero cíclico del ácido láctico) o su polímero polilactida. Sin embargo, esta opción no es rentable económicamente debido al alto coste de estos reactivos (Salerno y Domingo, 2014).

La esterificación entre el ácido láctico y el etanol es una reacción autocatalítica que está limitada termodinámicamente, alcanzándose bajas conversiones y unos productos

de baja pureza. Por este motivo, el uso de catalizadores es indispensable. Los catalizadores tradicionalmente utilizados han consistido en ácidos inorgánicos, como el ácido sulfúrico o el ácido clorhídrico. Sin embargo, debido a los problemas que genera su uso, se han sustituido por catalizadores ácidos heterogéneos, siendo las resinas de intercambio iónico las más utilizadas para este propósito (Pereira y col., 2011).

En la esterificación entre el ácido láctico y el etanol, la tradicional división entre la etapa de reacción y la etapa de separación de los productos implica el uso de altas cantidades de etanol para superar las limitaciones del equilibrio y alcanzar altas tasas de conversión, así como una mayor dificultad en la recuperación del lactato de etilo obtenido (Pereira y col., 2011). Como alternativa, se han propuesto varios procesos simultáneos de reacción-extracción, en los que las etapas de reacción y separación se combinan en una única etapa. Dos de estos procesos simultáneos consisten en la utilización de membranas (tanto mediante permeación de vapor, como pervaporación) (Pereira y col., 2010; Jafar y col., 2002) y en la destilación reactiva (Gao y col., 2007). Otro proceso consiste en una extracción reactiva, utilizando tolueno, éter etílico o benceno como fase extractiva (Wicki y Nielsen, 2005). Un proceso simultáneo estudiado, más novedoso, se basa en la utilización de un reactor de lecho móvil simulado (Pereira y col., 2009) y un reactor híbrido de membrana y lecho móvil simulado (Silva y col., 2010). Por último, se ha estudiado también la producción de lactato de etilo a través de la esterificación enzimática con lipasa B de *Candida antárctica*, con el objetivo de producir lactato de etilo enantioméricamente puro (Major y col., 2010).

Respecto a sus aplicaciones, el lactato de etilo se ha utilizado en distintos sectores de la industria, como por ejemplo, en el sector de la cosmética y perfumería, el farmacéutico o el alimentario. Además de su uso como aditivo aromatizante, el lactato de etilo se ha estudiado y utilizado fundamentalmente como disolvente. La Tabla 1.1 muestra algunas de las aplicaciones industriales del lactato de etilo.

Tabla 1.1. Usos y aplicaciones industriales del lactato de etilo.

Aplicación	Referencia
Disolvente en la industria relacionada con la fabricación de pinturas y recubrimientos de superficies	Pereira y col. (2011), Nikles y col. (2001)
Disolvente de limpieza en la industria del poliuretano, de sistemas electrónicos y de superficies metálicas	Henneberry y col. (2004)
Disolvente en la industria fitosanitaria	Pereira y col. (2014)
Excipiente de productos farmacéuticos y cosméticos	Greff (2000), Gupta (2006)
Disolvente en el tratamiento de contaminantes de suelos	Yap y col. (2015), Guo y col. (2010)
Medio de reacción en síntesis química	Wan y col. (2014), Dandia y col. (2013), Ghosh y col. (2013)

A este respecto, el lactato de etilo ha reemplazado industrialmente a otros disolventes como tolueno, acetona, 4-metil pentanona o xileno (Pereira y col., 2011), se utiliza en la disolución de recubrimientos para madera, poliestireno, metales y componentes magnéticos, en la disolución de compuestos con actividad pesticida y herbicida y en la limpieza de resinas del poliuretano, de componentes electrónicos, de superficies metálicas, entre otras. También se utiliza como excipiente en el sector farmacéutico y cosmético, mejorando la solubilidad de distintos compuestos bioactivos y actuando como vehículo en los tratamientos tópicos, al mejorar la penetración y liberación de dichos compuestos. Además, el lactato de etilo presenta propiedades anti acné al actuar como un agente sinérgico combinado con el ácido salicílico (Gupta, 2006; Wee y col., 2006) y se ha observado que podría incrementar el tiempo de protección frente a *Aedes aegypti* en formulaciones de repelentes de insectos (Drapeau y col., 2009). Igualmente, se ha estudiado el lactato de etilo como agente en el tratamiento de suelos contaminados en combinación con ciertos agentes quelantes, así como medio de reacción en la síntesis química de diversos compuestos.

Pese a ser un disolvente ampliamente utilizado en la industria, hay pocos estudios realizados respecto a su uso como disolvente de extracción de compuestos alimentarios, habiéndose estudiado principalmente para la extracción de compuestos de naturaleza lipídica (Tabla 1.2).

Tabla 1.2. Matrices alimentarias y compuestos bioactivos extraídos empleando lactato de etilo.

Compuesto	Matriz alimentaria	Referencia
Escualeno y α-tocoferol	Destilados de desodorización de aceite de oliva	Hernández y col., (2011) Vicente y col., (2011)
Esclareol	Salvia	Tombokan y col., (2008)
β-caroteno, luteína y licopeno	Zanahoria, maíz blanco y tomate	Ishida y Chapman (2009)
Licopeno	Subproductos de tomate	Strati y Oreopoulou (2011)
Astaxantina	*Xanthophyllomyces dendrorhous*	Wu y col., (2011)
Ácido γ-linolénico	Spirulina	Golmakani y col., (2012)
Compuestos fenólicos	Retama negra	Lores y col., (2015)

Hernández y col., (2011) y Vicente y col., (2011) estudiaron el fraccionamiento de materias primas lipídicas con lactato de etilo, con el objetivo de poder ser utilizado en la recuperación de tocoferol y escualeno, respectivamente, a partir de destilados de desodorización de aceite de oliva, debido a la miscibilidad parcial que presentan estos compuestos en lactato de etilo. Los autores estudiaron a distintas temperaturas el equilibrio líquido-líquido de mezclas de triglicéridos, el compuesto de interés (escualeno o α-tocoferol) y lactato de etilo, simulando así la composición de los destilados de desodorización. En este caso, el lactato de etilo mostró una buena selectividad en la extracción de ambos compuestos, especialmente a bajas temperaturas de extracción, por lo que podría ser utilizado en la recuperación de los mismos.

Por otra parte, Tombokan y col., (2008) estudiaron el comportamiento de fases del sistema ternario esclareol - lactato de etilo - CO_2, con el objetivo de determinar las mejores condiciones de operación en el diseño de procesos de extracción de dicho compuesto utilizando lactato de etilo y en su posterior recuperación mediante precipitación utilizando CO_2 como gas antisolvente para obtener así una fracción enriquecida en esclareol. A 35 ºC y en un rango de presiones entre 1,38 y 13,79 MPa los autores determinaron los mayores rendimientos de precipitación cuando se añadieron a la mezcla cantidades de CO_2 superiores al 30 % en peso y la presión aumentó por encima de 4,14 MPa.

Otros compuestos alimentarios estudiados han sido los carotenoides. Ishida y Chapman (2009) estudiaron la extracción de β-caroteno, luteína y licopeno (isómeros *trans* y *cis*) de zanahoria, maíz blanco y tomate liofilizados, respectivamente, utilizando lactato de etilo, a distintas temperaturas, y los resultados se compararon con los obtenidos utilizando etanol, acetato de etilo y cloruro de metileno / metanol / agua (40 / 40 / 20). El lactato de etilo fue más eficaz que el acetato de etilo y aunque las mayores cantidades de licopeno se extrajeron con la mezcla cloruro de metileno / metanol / agua, utilizando lactato de etilo se obtuvieron cantidades muy similares de licopeno y recuperaciones de los otros carotenoides entre el 65-80 % de la cantidad máxima extraída con dicha mezcla, por lo que el lactato de etilo podría reemplazar a la mezcla anterior, cuya toxicidad la hace no apta para su uso como disolvente de extracción en alimentos. Strati y Oreopoulou (2011) estudiaron la extracción de carotenoides procedentes de subproductos del tomate (semillas y piel), utilizando lactato de etilo y otros disolventes orgánicos (hexano, acetona, etanol y acetato de etilo). Con lactato de etilo se alcanzaron los mayores rendimientos de extracción de carotenoides en el intervalo de temperaturas estudiado (entre 25 y 70 ºC) y concentraciones de licopeno en el extracto entre 4 y 31 veces mayores que con el resto de disolventes. Wu y col., (2011) desarrollaron un método para extraer astaxantina de la levadura *Xanthophyllomyces dendrorhous*, en el que primero se realizó un tratamiento con un ácido orgánico para romper la pared celular, seguido de una etapa de extracción con disolventes. Los disolventes orgánicos estudiados fueron éter de petróleo, acetona, etanol, hexano y lactato de etilo, siendo este último el disolvente con el que se obtuvieron los mejores resultados. A temperatura ambiente y utilizando ácido láctico en la etapa de ruptura de la pared celular y mezclas lactato de etilo / etanol (1:1) en la etapa de extracción, se obtuvieron resultados muy similares a los obtenidos con los disolventes tradicionales DMSO (etapa de ruptura) y acetona (etapa de extracción).

Por otra parte, Golmakani y col., (2012) evaluaron el uso de lactato de etilo y etanol en la obtención de fracciones enriquecidas de ácido γ-linolénico procedente del microalga *Arthrospira platensis*. Se realizaron extracciones con líquidos presurizados, utilizando lactato de etilo, etanol y sus mezclas. La mayor recuperación del compuesto (74,7 %) se obtuvo a 180 ºC y 10 minutos de extracción utilizando la mezcla lactato de etilo / etanol (50 % v/v).

Por último, Lores y col., (2015) evaluaron el posible uso del lactato de etilo como disolvente de extracción de distintos compuestos fenólicos procedentes de retama negra (*Cytisus scoparius*). Para ello, se realizaron extracciones con líquidos presurizados a 120 ºC, utilizando como disolventes mezclas metanol / agua y lactato de etilo / agua (50 % v/v). Con ambos disolventes se obtuvieron concentraciones de compuestos fenólicos en el extracto y actividades antioxidantes similares, por lo que el lactato de etilo podría utilizarse en sustitución del metanol, un disolvente muy estudiado en la extracción de compuestos fenólicos procedentes de otras especies del género *Cytisus*, pero cuya toxicidad le hace no apto para ser utilizado en alimentos.

Una descripción más detallada acerca de la producción de lactato de etilo, de sus características físico-químicas y de sus aplicaciones, puede encontrarse en el capítulo titulado *"Ethyl lactate: a biorenewable agrochemical solvent for food tecnology"* del libro Handbook of Solvents (Volume 2: Use, Health, and Environment), recientemente publicado en el contexto del desarrollo de esta tesis.

1.3. TECNOLOGÍAS DE PRODUCCIÓN DE EXTRACTOS NATURALES

1.3.1. Extracción con líquidos presurizados

Las técnicas tradicionales de extracción sólido-líquido, como son la extracción Soxhlet, la extracción por reflujo o la maceración, generalmente requieren largos tiempos de extracción y altas cantidades de disolvente, obteniéndose extractos muy diluidos, que son más difíciles de tratar posteriormente, y rendimientos de extracción bajos en relación al consumo de disolvente (Richter y col., 1996).

En los últimos años se han desarrollado nuevas técnicas para tratar de solucionar estos problemas. Una de ellas consiste en la extracción con líquidos presurizados (*Pressurized Liquid Extraction*, PLE), que consiste en una extracción sólido-líquido a temperaturas más elevadas que las utilizadas en las técnicas tradicionales, a través de la aplicación de presiones lo suficientemente altas para mantener el disolvente en estado líquido, permitiendo así utilizar temperaturas de extracción por encima de su punto de ebullición normal (Carabias-Martínez y col., 2005).

El efecto de aplicar temperaturas de extracción elevadas se traduce en un mayor rendimiento de extracción, en tiempos de extracción más cortos y en un menor consumo de disolventes, debido principalmente a la mayor solubilidad de los solutos, mayor ruptura de las interacciones soluto-matriz y soluto-soluto y menor viscosidad del disolvente que se alcanza al aumentar la temperatura, produciéndose un mejor contacto disolvente-soluto y generándose mayores velocidades de difusión. Estas son las principales ventajas respecto a las técnicas de extracción tradicionales, en las cuales la temperatura de extracción máxima es la temperatura de ebullición del disolvente (Richter y col., 1996).

La temperatura es, por tanto, el parámetro fundamental en la extracción con líquidos presurizados. El objetivo principal al aplicar presión es mantener el disolvente en estado líquido durante la extracción y pese a no ser un parámetro determinante, la presión tiene un efecto positivo en la extracción, favoreciendo la penetración del disolvente en los poros de la matriz y mejorando el contacto entre disolventes y solutos, beneficiando así la extracción (Carabias-Martínez y col., 2005).

La extracción con líquidos presurizados es también muy conocida en la bibliografía como extracción acelerada con disolventes (*Acelerated Solvent Extraction*, ASE). Otros nombres utilizados son extracción con disolventes a alta presión (HPSE) o extracción con disolventes a alta temperatura y a alta presión (HPHTSE). Cuando el disolvente de extracción utilizado es agua, la técnica se denomina extracción con agua subcrítica (SWE), extracción con agua caliente (HWE), extracción con agua caliente presurizada (PHWE) o extracción con agua a alta temperatura (HTWE), para diferenciarla respecto al uso de disolventes orgánicos (Richter y col., 1996).

A temperaturas elevadas, el agua presenta unas propiedades físico-químicas distintas a las del agua a temperatura y presión ambiente, especialmente en lo relativo a su constante dieléctrica. Así, un aumento de la temperatura bajo presiones moderadas produce una disminución de su constante dieléctrica. A temperatura y presión ambiente, el agua es un disolvente polar con una constante dieléctrica alta. Sin embargo, a 300 ºC y 23 MPa su valor decrece hasta valores cercanos a los del etanol, metanol o la acetona a 25 ºC, por lo que la polaridad del agua disminuye con la temperatura, siendo posible su uso en sustitución de disolventes orgánicos en la extracción de compuestos de polaridad media o baja (Turner e Ibáñez, 2012; Teo y col., 2010; Carabias-Martínez y col., 2005).

Inicialmente, la extracción con líquidos presurizados se utilizó en la extracción de contaminantes presentes en suelos, sedimentos y lodos procedentes de aguas residuales. Sin embargo su uso se extendió a otras áreas, siendo actualmente una técnica aplicada también en la extracción de compuestos procedentes de matrices alimentarias (Ibañez y col., 2003). En este caso, la extracción con líquidos presurizados se ha aplicado en la obtención de numerosos compuestos bioactivos, como por ejemplo, aceites esenciales procedentes de tomillo, orégano, albahaca o cúrcuma; antioxidantes de microalgas y de manzana, flavonoides de cebolla y espinacas, antocianinas de fresas y de la piel de uvas tintas, catequinas y proantocianidinas de semillas de uva, ácidos fenólicos de subproductos de la patata, oleuropeína de hojas de olivo, ácido rosmarínico y ácido carnósico de romero, isoflavonas de soja, carotenoides de subproductos de la zanahoria, β-sitosterol de nueces, lignanos de semillas de lino o capsaicina de pimientos, entre otros (Herrero y col., 2013; Teo y col., 2010).

1.3.2. Tecnología de fluidos supercríticos

La tecnología de fluidos supercríticos comenzó a desarrollarse hace unos cincuenta años y se ha convertido en una tecnología ecológica y eficaz para el procesamiento de productos naturales y el desarrollo de nuevos procesos alimentarios.

En la Figura 1.3, en cualquier punto sobre la curva que va desde el punto triple al punto crítico, el cambio de fase se produce manteniendo una interfase definida entre la fase líquida y el vapor en equilibrio. Es decir, la evaporación o condensación de una sustancia pura está asociada a un cambio brusco de entalpía y densidad. Por encima del punto crítico, este cambio no se produce, generándose una única fase. Por tanto, se puede definir el punto crítico como aquel por encima del cual no se produce la licuefacción de un gas al presurizar a temperatura constante, y una sustancia pura es denominada fluido supercrítico cuando su presión y temperatura son superiores a los valores de su presión y temperatura críticas.

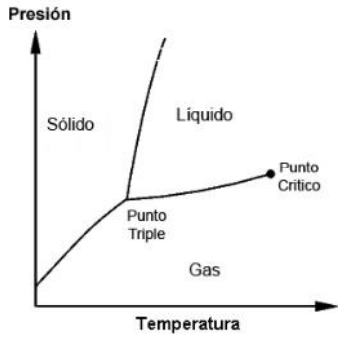


Figura 1.3. Diagrama de fases de una sustancia pura.

Como se muestra en la Tabla 1.3, los fluidos supercríticos (SF) exhiben comportamientos y propiedades físicas intermedias entre las de los líquidos y los gases. Las densidades son similares a las de los líquidos y las propiedades de transporte más similares a las de los gases. Sin embargo, esta característica no se presenta cuando la temperatura es mucho más alta que la crítica, ya que en esas condiciones la densidad es mucho menor que la densidad crítica. Por ello, un fluido supercrítico también lo podemos definir como aquella sustancia cuya temperatura y presión son más altas que sus valores críticos y su densidad es similar o mayor que su densidad crítica (Darr y Poliakoff, 1999).

Debido a sus propiedades, el principal fluido supercrítico utilizado es el dióxido de carbono (CO_2). Entre ellas destacan su baja temperatura crítica (31,1 ºC), una presión crítica moderada (7,38 MPa), no ser tóxico, no ser corrosivo ni inflamable, ser relativamente económico y haber sido reconocido como GRAS.

Tabla 1.3. Densidad, viscosidad y difusividad media de gases, líquidos y fluidos supercríticos.

Fluido	Densidad ($g \cdot mL^{-1}$)	Viscosidad ($g \cdot cm^{-1} \cdot s^{-1}$)	Difusividad ($cm^2 \cdot s^{-1}$)
Gas	$(0,1 - 2) \times 10^{-3}$	$(0,5 - 3,5) \times 10^{-4}$	0,01 - 1
SF	0,2 - 1	$(0,2 - 1) \times 10^{-3}$	$(3,3 - 0,1) \times 10^{-4}$
Líquido	0,6 - 1,6	$(0,5 - 3) \times 10^{-2}$	$(0,2 - 2) \times 10^{-5}$

Estas propiedades intermedias entre las de un líquido y un gas, sumado a los pronunciados cambios de densidad y viscosidad que se producen en condiciones cercanas al punto crítico, y la posibilidad de modular estos parámetros con moderadas variaciones de presión y temperatura, hacen que el uso de los fluidos supercríticos sea interesante en varios procesos, como por ejemplo, en la extracción y fraccionamiento de materias primas líquidas y sólidas, para controlar comportamientos de fase en procesos de separación, para diseñar la morfología de partículas en el procesado de materiales o para controlar velocidades de reacción en procesos de síntesis enzimática (Perrut, 2000).

Respecto a su uso en procesos de extracción, la tecnología de fluidos supercríticos ha alcanzado aplicaciones industriales importantes en el campo de los alimentos, tales como la producción comercial de café y té descafeinado, la extracción de lúpulo, aceites esenciales, especias y aromas o la extracción de tricloroanisoles del corcho, entre otras (Perrut, 2000; MacHugh y Krukonis, 1994).

La extracción con fluidos supercríticos (*Supercritical Fluid Extraction*, SFE) se caracteriza por la posibilidad de ajustar el poder solvente del fluido supercrítico modificando la temperatura y presión, haciendo así que el fluido pueda disolver y extraer de forma selectiva distintas clases de compuestos. La presión y la temperatura también tienen influencia en la viscosidad del fluido supercrítico, la cual disminuirá al aumentar la temperatura y disminuir la presión. Los fluidos supercríticos presentan viscosidades cien veces menores y coeficientes de difusión un orden de magnitud superior a los de los líquidos. Este efecto, unido a una tensión superficial despreciable,

resulta en una gran penetración del fluido en matrices sólidas y altas velocidades de transferencia del soluto en el fluido supercrítico (Brunner, 2005).

Otra de las ventajas de los procesos SFE es la posibilidad de obtener productos finales libres de disolvente. Los compuestos solubilizados en el fluido supercrítico pueden separarse del disolvente mediante despresurización. Al reducirse drásticamente la presión, los compuestos dejan de ser solubles y precipitan. Del mismo modo se puede realizar un fraccionamiento de los compuestos extraídos mediante precipitación selectiva, variando las condiciones de presión y/o temperatura. (Vicente y col., 2012).

Respecto al dióxido de carbono como fluido supercrítico, además de las ventajas anteriormente mencionadas, su baja temperatura crítica (31,1 ºC) permite procesar materiales térmicamente inestables, como son la mayoría de productos naturales y hacerlo en condiciones no oxidantes.

El principal inconveniente que presenta el dióxido de carbono supercrítico ($SCCO_2$) como disolvente es su baja polaridad, lo que dificulta la solubilización de compuestos polares. Así, se utilizan cosolventes para aumentar la polaridad del fluido supercrítico como consecuencia de las distintas interacciones que se producirán entre dióxido de carbono – cosolvente – soluto, aumentando también la densidad del fluido supercrítico y así su capacidad de solvatación. Entre los disolventes orgánicos utilizados se encuentran el metanol, octano, acetonitrilo, hexano y acetona, aunque por cuestiones de toxicidad, los cosolventes más estudiados en extracción de alimentos son el agua y el etanol (Foster y col., 1993; Dobbs y col., 1987).

Se han llevado a cabo numerosos estudios acerca de la aplicación de los fluidos supercríticos en la extracción de compuestos alimentarios. La investigación básica y el desarrollo de nuevas aplicaciones se mantienen intensamente activos, particularmente en el campo de la extracción de fitoquímicos a partir de matrices vegetales. Distintos compuestos fenólicos, carotenoides y aceites fijos y aceites esenciales, entre otros compuestos, se han obtenido mediante extracción con fluidos supercríticos a partir de un gran número de matrices vegetales (Herrero y col., 2013; Pereira y Meireles, 2010).

Por otro lado, los fluidos supercríticos también pueden utilizarse en procesos de secado, formación de partículas y encapsulado de compuestos de interés en matrices poliméricas. Debido a las propiedades físico-químicas de los fluidos supercríticos, el

proceso de formación de partículas permite obtener tamaños de partícula menores (escala micrométrica y nanométrica) y de una forma más controlada que mediante los procesos tradicionales. Esto es importante, ya que muchos compuestos bioactivos de interés alimentario son poco solubles o insolubles en disoluciones acuosas, y un menor tamaño de partícula puede favorecer su incorporación a un alimento. Por otro lado, esto podría promover una mayor absorción y biodisponibilidad del ingrediente activo (Cocero y col., 2009).

Las técnicas tradicionales para la formación de micro y nano-partículas, como por ejemplo, el secado por atomización, la micronización mecánica, la coacervación, la recristalización, la inclusión en liposomas, la liofilización o la polimerización interfacial, entre otras, presentan varios inconvenientes, como son la degradación térmica de los solutos como consecuencia de aplicar altas temperaturas, alteraciones en la estructura del producto (como ocurre en el caso de la liofilización, en la que se pueden generar poros en la estructura de la partícula formada), el uso de altas cantidades de disolventes orgánicos, la presencia de altas cantidades residuales de disolvente difíciles de eliminar, fenómenos electrostáticos y, en general, dificultad para controlar el tamaño de partícula y la distribución de tamaños de partícula durante el proceso, obteniéndose productos poco homogéneos, de tamaños irregulares (Gadkari y Balaraman, 2015; Rodríguez-Meizoso y Plaza, 2015).

En cambio, debido a las propiedades físico-químicas de los fluidos supercríticos, con altas velocidades de difusión de los solutos, se producen rápidamente sistemas supersaturados y una precipitación rápida de los solutos en forma de pequeñas partículas, así como una nucleación y crecimiento homogéneo de los cristales que conlleva una mejora en las características de las partículas obtenidas (Reverchon y Adami, 2006). A estas ventajas hay que añadir otras ya mencionadas anteriormente para la extracción supercrítica, como son la posibilidad de llevar a cabo el proceso sin necesidad de utilizar disolventes orgánicos, la posibilidad de usar bajas temperaturas, así como realizarse en un ambiente reductor, importante cuando están presentes solutos sensibles a la degradación térmica y oxidativa.

Se han diseñado y patentado numerosos procesos de formación de partículas con fluidos supercríticos y la mayoría de ellos están basados en tres técnicas principales (Figura 1.4), que pueden clasificarse de acuerdo con la función que ejerza el fluido

supercrítico, generalmente el dióxido de carbono. Por un lado, el fluido supercrítico puede actuar como un disolvente, mecanismo que corresponde al proceso denominado RESS (*Rapid Expansion of Supercritical Solutions*). Por otro lado, el fluido supercrítico puede actuar como un antisolvente, procedimiento representado por el proceso SAS (*Supercritical Antisolvent*). En tercer lugar, el fluido supercrítico puede actuar como un soluto o un medio dispersante, representado por el proceso PGSS (*Particles from Gas Saturated Solutions*) (Rodríguez-Meizoso y Plaza, 2015). En estos procesos, el tamaño, composición y morfología de las partículas obtenidas depende de diversos factores, como son la estructura química del material a precipitar, parámetros del proceso como son la presión, la temperatura o flujos, y el diseño del equipo, como por ejemplo, las dimensiones de la cámara de precipitación o la geometría de la boquilla o el restrictor utilizado.

En el proceso RESS (Figura 1.4a), el fluido supercrítico es bombeado hacia una unidad de extracción a las condiciones deseadas de presión y temperatura, donde se encuentra el soluto de interés, el cuál será disuelto por el fluido supercrítico y formará un aerosol a partir de la disolución resultante, al hacerla pasar a través de una boquilla situada en una unidad de precipitación a baja presión. Esta rápida despresurización de la disolución a través de la boquilla, producirá una rápida y homogénea supersaturación, que originará una rápida nucleación de los solutos en forma de pequeñas y homogéneas partículas que precipitarán, separándose así de la corriente de gas.

El principal inconveniente de este proceso es que se encuentra restringido a sustancias que presentan una alta solubilidad en el dióxido de carbono supercrítico, por lo que su aplicación se limita a solutos de baja polaridad y/o solutos que no presenten un tamaño molecular demasiado elevado. Para solucionar este problema, se pueden utilizar cosolventes, pero esto a veces no es factible, ya que la solubilidad del precipitado en el cosolvente a condiciones de presión y temperatura ambiente no debería ser elevada para evitar la disolución de las partículas precipitadas, pero, por otro lado, debe ser lo suficientemente alta en las condiciones del proceso RESS para que el fluido supercrítico disuelva los solutos de interés. Además, al usar un cosolvente se debe añadir una etapa posterior de recuperación del precipitado, lo que dificultaría e incrementaría el coste del proceso (Wediner, 2009; Martín y Cocero, 2008; Jung y Perrut, 2001).

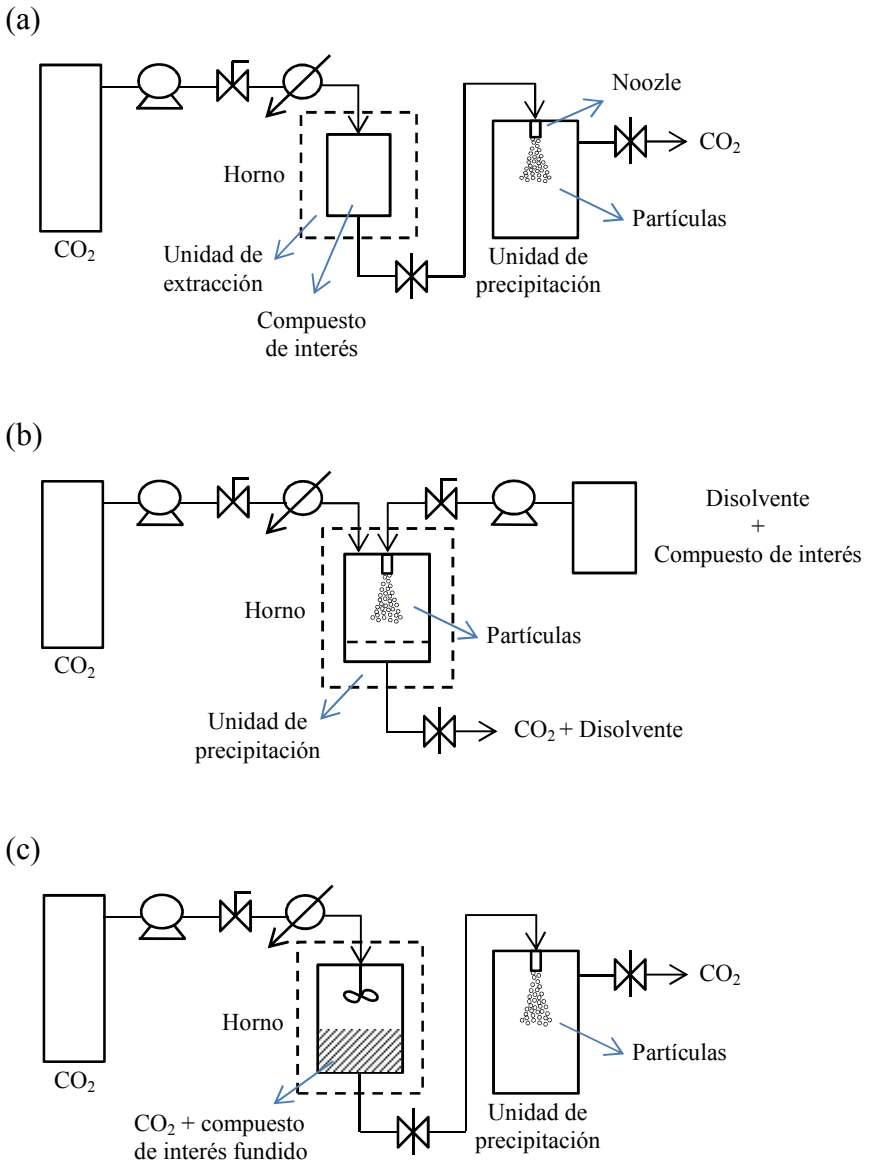


Figura 1.4. Principales procesos de formación de partículas utilizando fluidos supercríticos: (a) proceso RESS, (b) proceso SAS, (c) proceso PGSS.

En el proceso SAS (Figura 1.4b) se aprovecha la ventaja de la baja solubilidad de ciertos solutos en CO_2. El soluto es inicialmente disuelto en un disolvente orgánico y la precipitación de las partículas se produce debido a la reducción de la solubilidad como consecuencia de la adición a la disolución de CO_2, que actúa como antisolvente. La saturación de la disolución orgánica con CO_2 produce una disminución de la capacidad de disolución del disolvente líquido. De este modo, se produce la supersaturación de la

disolución, las partículas precipitan y se separan de la mezcla formada por el disolvente y el fluido supercrítico.

Una desventaja respecto al proceso RESS es que, normalmente, la supersaturación producida por el antisolvente no es tan grande como la producida por la despresurización en el proceso RESS, por lo que el tamaño de las partículas producidas mediante el proceso SAS es mayor que las producidas mediante RESS (Martín y Cocero, 2008; Jung y Perrut, 2001).

Básicamente, el proceso SAS puede llevarse a cabo en forma discontinua o semi-continua. En el primer caso, el disolvente orgánico con el soluto se encuentran inicialmente en el interior de la cámara de precipitación y se introduce el fluido supercrítico durante un período lo suficiente largo como para lograr la disolución total del disolvente orgánico en la fase supercrítica. En el segundo caso, tanto el fluido supercrítico, como la disolución con el compuesto de interés, son bombeados al interior de la cámara de precipitación de forma continua y simultánea, hasta que finalmente las partículas precipitadas y retenidas en el interior de la cámara de precipitación son recogidas tras la despresurización del sistema. La disolución es atomizada al hacerla pasar a través de una boquilla a la entrada de la cámara de precipitación, donde se pondrá en contacto con en el fluidos supercrítico, produciéndose el efecto antisolvente. A este proceso se le ha denominado ASES (*Aerosol Solvent Extraction System*) (Martín y Cocero, 2008; Jung y Perrut, 2001).

Otra denominación muy común del proceso SAS es GAS (*Gas Anti Solvent*). Aunque no hay un consenso al respecto, se puede nombrar al proceso como GAS cuando el antisolvente se encuentra por debajo de su presión crítica, mientras que el proceso SAS utiliza un fluido en condiciones supercríticas (Martín y Cocero, 2008).

Se ha patentado un considerable número de variantes del proceso SAS. En el caso de los procesos semi-continuos, una de las principales diferencias entre los distintos diseños lo constituye la boquilla, con el objetivo de mejorar el contacto entre la disolución y el antisolvente y obtener menores tamaños de partícula. Una variante la constituye el proceso denominado SEDS (*Enhanced Dispersion by Supercritical Fluids*). La diferencia principal de este proceso es la utilización de una boquilla coaxial, por donde circula el CO_2 por un lado y la disolución por el otro, lo que provoca que la disolución y el antisolvente se pongan en contacto entre sí antes de entrar a la unidad de

precipitación, por lo que la dispersión (la mayor velocidad del CO_2 produce la atomización de la disolución en pequeñas gotas) y la extracción del disolvente por el fluido supercrítico se producen simultáneamente (Martín y Cocero, 2008).

Por último, el proceso PGSS™ (Figura 1.4c), donde el fluido supercrítico actúa como medio dispersante, también se realizan en dos etapas, como en el caso del proceso RESS. Primero, el dióxido de carbono supercrítico se solubiliza en el soluto, el cual funde cuando es presurizado (el soluto tiene que estar fundido o ser un líquido), aprovechando la alta solubilidad de los gases comprimidos en los líquidos, formando una disolución saturada de gas, la cual se expande posteriormente a través de la boquilla en una unidad de despresurización. La expansión del gas produce una ruptura del líquido en pequeñas gotas, una reducción de la solubilidad y una intensa reducción de la temperatura, siendo este enfriamiento la principal fuerza impulsora de la supersaturación, que como resultado originará la solidificación de los compuestos fundidos y la formación de partículas sólidas. Además del efecto producido por el enfriamiento, el fluido supercrítico actúa como un dispersante aumentando la atomización de la disolución al expandirse durante la despresurización (Martín y Cocero, 2008).

También en este caso el proceso puede llevarse a cabo de forma discontinua, introduciendo el fluido supercrítico en una autoclave donde se encuentra la disolución y, de forma continua, donde ambos, fluido supercrítico y disolución, son bombeados y puestos en contacto en un mezclador estático antes de formarse el aerosol y ser despresurizados (Jung y Perrut, 2001).

El proceso PGSS™ puede llevarse a cabo con un menor consumo de CO_2 y a menor presión que las otras técnicas, y puede formar partículas a partir de compuestos que no necesariamente tienen que ser solubles en CO_2, debido a que se produce la expansión de la disolución del soluto, y no únicamente de la fase supercrítica, como en el caso del proceso RESS. No obstante, como el soluto sometido a precipitación debe estar fundido antes de producirse la expansión del gas, el proceso PGSS™ no es adecuado en el caso compuestos sensibles al calor. En cambio, la técnica es especialmente apropiada para sustancias como polímeros y ceras, en las cuales la disolución de CO_2 origina una reducción considerable del punto de fusión de estas sustancias y al producirse la

despresurización, el efecto de enfriamiento se combina con el incremento del punto de fusión del soluto.

Una desventaja del proceso PGSS™ es que la supersaturación se produce por enfriamiento, que es la fuerza impulsora de la precipitación, pero esta supersaturación es mucho menor que la supersaturación alcanzada en el proceso RESS, debido a la perturbación mecánica que se genera al despresurizar la mezcla a través de la boquilla, y que en el proceso SAS, debido al efecto antisolvente. Por ello, las partículas obtenidas con el proceso PGSS™ generalmente son mayores que las obtenidas con RESS o SAS (Martín y Cocero, 2008).

Estos tres principales procesos supercríticos de formación de partículas se han utilizado para producir micro- y nano-partículas a partir de distintos ingredientes alimentarios de naturaleza muy diversa. Por ejemplo, el proceso RESS se utilizó para obtener partículas de astaxantina, fitosteroles y antiocianinas; mientras que quercetina, proteínas, ácido nicotínico, luteína, β-caroteno y lecitina se obtuvieron mediante la técnica SAS; y ácido benzoico y fosfolípidos, entre otros, mediante el procedimiento PGSS™ (Rodríguez-Meizoso y Plaza, 2015; Knez y Weidner, 2003; Jung y Perrut, 2001).

1.4. FUENTES VEGETALES QUE CONTIENEN CAFEÍNA

1.4.1. Café

El café es la bebida obtenida mediante infusión a partir de las semillas tostadas y molidas de los frutos de la planta de cafeto (género *Coffea*) (Figura 1.5), perteneciente a la familia Rubiaceae. Aunque se han identificado más de 80 especies de café a nivel mundial, solo dos son económicamente importantes: la variedad Arábica (*Coffea arabica*) y la variedad Robusta (*Coffea canephora*) (Farah, 2012). La variedad Arábica es la más producida (se estima que el 55 % de la producción corresponderá a Arábica, respecto al 45 % de Robusta, para la temporada 2014/15), siendo Brasil el mayor productor y exportador, principalmente de Arábica. No obstante, la producción de Arábica ha ido descendiendo en los últimos cuatro años, mientras que la producción de Robusta ha experimentado un ascenso continuo (USDA, 2014).

La planta de café se desarrolla en regiones tropicales y ecuatoriales, siendo Brasil, Vietnam, Colombia, Indonesia y América Central, junto con Méjico, los mayores productores a nivel mundial. En el caso de la Arábica, su cultivo es más delicado, menos productivo y se localiza principalmente en zonas altas de montaña. Los principales países productores de esta variedad son Brasil, Colombia, Etiopía y Honduras. Respecto a la variedad Robusta, tolera mayores temperaturas y se adapta con mayor facilidad a distintos ámbitos, pudiendo crecer a baja altitud con rendimientos más elevados y obteniéndose, por lo general, un café con menor calidad (dos Santos y col., 2015). Los principales países productores de esta variedad son Vietnam, Brasil, Indonesia, India y Uganda (USDA, 2014).

(a) (b) (c)

Figura 1.5. (a) Planta de café, (b) fruto de la planta de café, (c) semilla del fruto

En la elaboración del café se llevan a cabo una serie de etapas, como se muestra en la Figura 1.6. Junto con las diferencias debidas a las características intrínsecas de cada variedad y las condiciones de cultivo (temperatura, humedad, propiedades del suelo, etc.), la recolección y el resto de etapas implicadas en el procesado del café van a tener influencia en la calidad final del producto (Kleinwachter y col., 2014).

La primera etapa consiste en la recolección, que se puede realizar de forma manual, seleccionando y recogiendo solo los frutos maduros y obteniendo un producto homogéneo y de mejor calidad. También, se puede realizar mediante el llamado despalillado, tanto mecánica como manualmente, donde se raspan las ramas donde está el fruto y se recolectan todos, independientemente de su madurez y, por último, la recolección se puede llevar a cabo de forma mecanizada, agitando el árbol y recogiendo el fruto (Kleinwachter y col., 2014).

Tras la recolección del fruto se produce la eliminación de las capas externas del fruto, ya que el fruto de café o, también llamado, cereza o baya de café, contiene distintas capas de tejido que hay que eliminar para obtener la semilla o grano de café verde. La obtención del café verde puede realizarse mediante dos tipos de procesado: el procesado por vía húmeda y el por procesado vía seca (Figura 1.6).

En el procesado por vía húmeda, las cerezas se introducen en un tanque de agua para separar los frutos enfermos y defectuosos (flotantes) de los sanos y desarrollados (hundidos). Después se produce el despulpado, que consiste en la eliminación de la piel exterior (epicarpio) y parte de la pulpa (mesocarpio) del fruto aplicando presión, dejando al descubierto el grano recubierto con el pergamino (endocarpio), el cual está recubierto a su vez por una parte del mesocarpio llamada mucílago. El mucílago se degrada mediante fermentación en tanques, en una etapa denominada desmucilaginado. Tras ella se produce el secado (al sol o en secaderos) del pergamino y de la capa inferior (tegumento) hasta alcanzar alrededor de un 12 % de humedad, y después el café pergamino se almacena hasta justo antes de venderse, momento en el que se lleva a cabo la eliminación de esas capas. La eliminación del pergamino y del tegumento se realiza en la etapa final de descascarillado, obteniendo, por lo general, dos granos (endospermo) de café verde por cada fruto procesado (Kleinwachter y col., 2014; Esquivel y Jiménez, 2012).

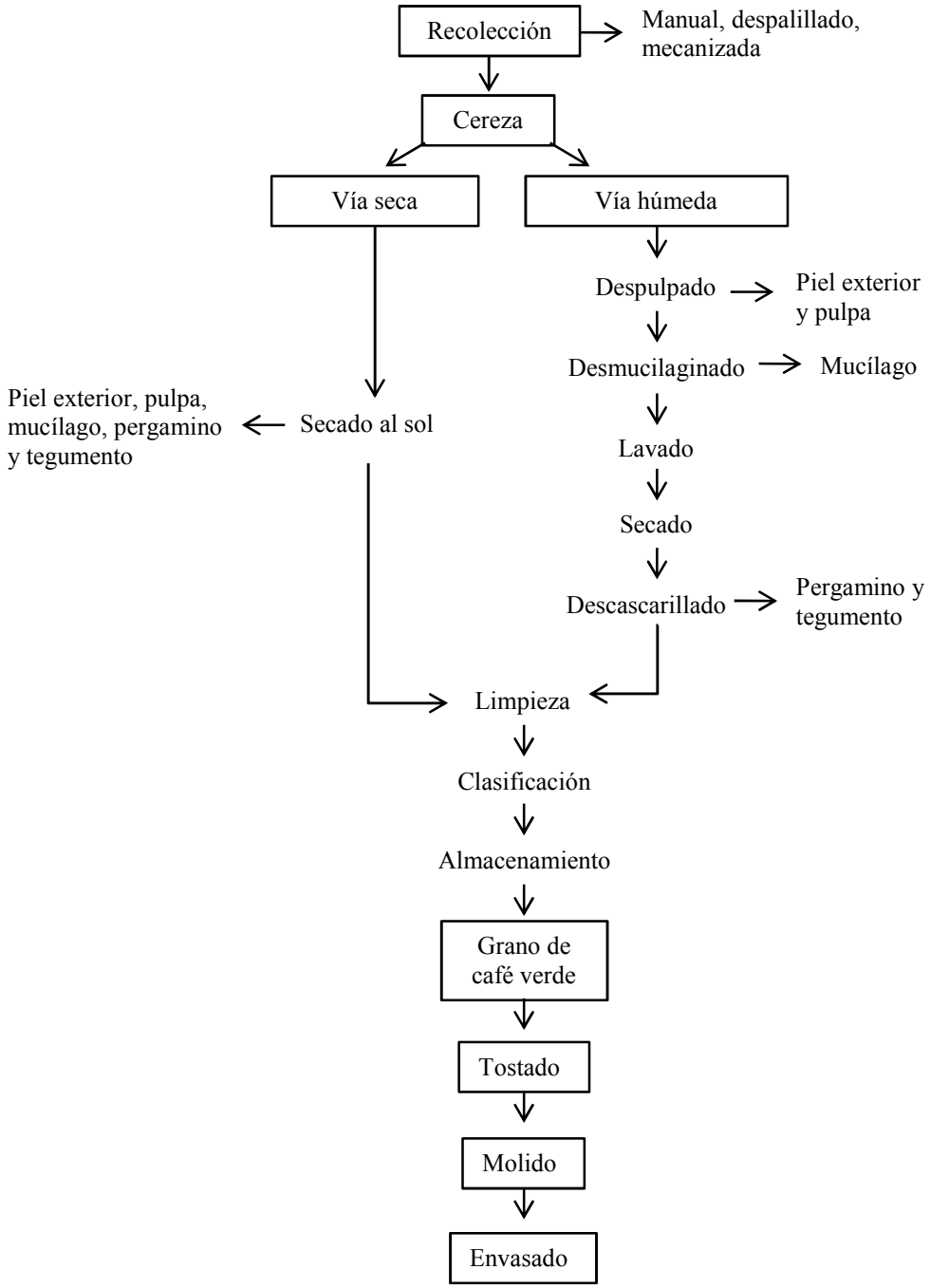


Figura 1.6. Esquema general del procesado del fruto de la planta de cafeto en la producción de café.

En el caso del procesado por vía seca (método más antiguo y sencillo), también se produce una etapa inicial de limpieza, aventando o sumergiendo en agua el fruto, para eliminar hojas y suciedad y para separar las cerezas maduras de las demasiado maduras y dañadas. Después, el fruto se expone a un secado al sol o en secaderos (en el caso de grandes plantaciones) hasta alcanzar aproximadamente un 10-12 % de humedad. Tras el

secado, se lleva a cabo una etapa de descascarillado en la que se quitan de una sola vez las capas exteriores del grano (Farah, 2012; Esquivel y Jiménez, 2012; Clarke, 2003[a]).

En el caso de la variedad Arábica, suele aplicarse el procesado por vía húmeda, mientras que el procesado por vía seca se aplica tanto a cafés de la variedad Robusta, como de la variedad Arábica (principalmente a los producidos en Brasil). Las materias primas recolectadas de diferentes formas y, sobre todo, el efecto que ejerce sobre el grano el aplicar dos procesos distintos entre sí, produciendo variaciones en el metabolismo y en la progresión de la germinación del grano, tienen una gran influencia en la calidad final del producto (Kleinwachter y col., 2014).

Una vez obtenido el café verde, la siguiente etapa en la elaboración es el tostado, que se lleva a cabo entre 200-240 ºC en, generalmente, un tiempo menor de 12 minutos (Farah, 2012; Clarke, 2003[b]). Durante esta etapa, se reduce el contenido de humedad y el peso de los granos (una reducción incluso mayor del 21 % en tuestes más intensos) y se produce una expansión en su volumen, debido fundamentalmente a la liberación de agua y dióxido de carbono. Igualmente, se formarán pigmentos que harán que los granos adquieran un color marrón característico, más oscuro cuanto mayor sea el grado de tueste, y se desarrollarán los aromas y sabores característicos del café (Oestreich-Janzen, 2010). Las reacciones químicas que se producen son complejas, siendo la reacción de Maillard la prevalente, junto con otras como la degradación de Strecker.

La fracción de compuestos volátiles del café verde (principalmente alcoholes, ésteres y aldehídos) tiene poca importancia cualitativa y cuantitativa en las características sensoriales del café, siendo la fracción de no volátiles los principales compuestos que actúan como precursores en el desarrollo organoléptico que se produce en el café durante la etapa de tostado (Farah, 2012). Los principales compuestos del café verde que participan en estas reacciones son polisacáridos, oligosacáridos (principalmente sacarosa), aminoácidos libres, ácidos clorogénicos y el alcaloide trigonelina, así como los compuestos formados a partir de la fragmentación de carbohidratos y proteínas, produciéndose una serie de reacciones de condensación, rotura, reordenamiento, pirólisis y polimerizaciones que conducirán a la formación de los pigmentos y compuestos volátiles característicos (pirazinas, oxazoles, pirroles, pirimidinas, furanos, lactonas o distintos fenoles y melanoidinas). En cambio, otros

compuestos, como cafeína y el aceite, prácticamente no se van a ver afectados (Kleinwachter y col., 2014; Oestreich-Janzen, 2010; Clarke, 2003[b]).

En el café verde, la fracción de compuestos no volátiles está formada principalmente por agua, polisacáridos, proteínas y aminoácidos libres, lípidos, minerales, ácidos orgánicos, ácidos clorogénicos, cafeína y trigonelina. El contenido de agua es de alrededor de 8,5-12 % y los polisacáridos suponen más de la mitad del peso seco en ambas variedades. En el caso de las proteínas (10-15 % peso seco), la variedad Robusta presenta cantidades ligeramente mayores que la Arábica, mientras que la cantidad de lípidos (7-15 % peso seco) es alrededor de dos veces mayor en la variedad Arábica (Farah, 2012; Oestreich-Janzen, 2010; Clarke, 2003[a]).

De los compuestos encontrados en el grano de café verde, la trigonelina (Hamden y col., 2013); los diterpenos (cuya cantidad supone hasta el 20 % de los lípidos totales), principalmente cafestol y kahweol (Araújo y Sandi, 2006); los ácidos clorogénicos (6-14 % y 4-8 % en peso en la variedad Robusta y Arabica, respectivamente) (Yan y col., 2015; Farah, 2012; Alonso-Salces y col., 2009) y la cafeína (Griffiths y col., 1990), son los principales compuestos bioactivos, los cuales, a su vez, son también importantes en el desarrollo de las características sensoriales durante el tueste, por lo que la cantidad de alguno de estos compuestos disminuirá respecto al grano de café verde, como ocurre con la trigonelina, los diterpenos (de forma no tan pronunciada) y los ácidos clorogénicos (Farah, 2012; Oestreich-Janzen, 2010; Clarke, 2003[b]).

Se han descrito varias actividades biológicas asociadas a los ácidos clorogénicos, como, por ejemplo, actividad antimicrobiana, antiviral, antifúngica, antioxidante y anticarcinogénica (Suárez-Quiroz y col., 2013; Morishita y Ohnishi, 2001). Durante el tueste, estos compuestos participan en la generación del color, sabor y aroma del café, confiriendo astringencia, amargor y acidez a la bebida (Farah, 2012). Debido a su inestabilidad térmica, la cantidad de ácidos clorogénicos se reduce en más del 50 % en tostados de intensidad media (Clark, 2003[b]) y se degrada prácticamente por completo (cantidades menores del 5 %) cuando los granos son sometidos a unas condiciones de tueste más intensas, siendo precursores en la formación de diversos derivados fenólicos y melanoidinas, entre otros (Ludwig y col., 2014; Budryn y col., 2009; Farah y Marino Donangelo, 2006).

Otro metabolito con actividad biológica presente en el café es la cafeína (Figura 1.7), una metilxantina (1,3,7-trimetil-xantina) que actúa como antagonista de los receptores de adenosina. La concentración de cafeína en el grano de café verde de la variedad Arábica es de alrededor del 1 % en peso seco (0,9-1,3 %), mientras que en la variedad Robusta, su cantidad puede llegar a ser hasta dos veces mayor (1,5-2,5 %) (Farah, 2012; Oestreich-Janzen, 2010).

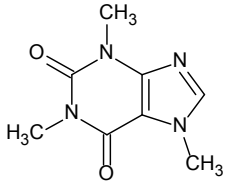


Figura 1.7. Estructura química de la cafeína.

Desde un punto de vista organoléptico, la cafeína es responsable de parte del amargor de la bebida, ya que su concentración en el grano no se altera significativamente durante el proceso de tueste (Oestreich-Janzen, 2010; Clarke, 2003[b]). Por otro lado, la cafeína ha sido intensamente estudiada desde un punto de vista biológico, debido a las diferentes actividades funcionales que se han asociado a ella como consecuencia de su consumo. No obstante, existe una gran controversia respecto a sus efectos sobre la salud.

Por un lado, se han descrito efectos positivos derivados del consumo de cafeína, como, por ejemplo, un aumento de la actividad y de la capacidad de concentración, disminución de la fatiga o un aumento de la memoria a corto plazo (Glade, 2010; Peeling y Dawson, 2007). Combinada con otros compuestos, como la ergotamina, podría aplicarse para el tratamiento de migrañas y como analgésico administrado junto con aspirina (Kumar y Ravishankar, 2009; Tfelt-Hansen y col., 2000). La inhibición débil de la actividad fosfodiesterasa por parte de la cafeína produce relajación de la tráquea y el tubo bronquial, por lo que tiene un efecto favorable en el tratamiento de los ataques de asma (Debry, 1994). Igualmente, la cafeína ha mostrado tener propiedades antioxidantes (Kronschläger y col., 2013; Paston y Tarasov, 2011; Devasagayam y col., 1996; Stadler y col., 1996). Existen publicados numerosos trabajos relacionados con el estudio del efecto de las condiciones de extracción y del tueste en las características antioxidantes del extracto obtenido, y a la cafeína se le ha atribuido parte de las propiedades antioxidantes de la infusión (Ludwig y col., 2012; Vignoli y col., 2011).

Por otro lado, la cafeína, pese a ser generalmente bien tolerada y tener un efecto positivo en el aumento de la capacidad de concentración y el rendimiento físico cuando se consume de forma moderada (Farah, 2012), también puede causar efectos adversos a pequeñas dosis tras ser ingerida, hasta ser metabolizada (2-6 horas). Por ejemplo, ocasionar aumentos de presión arterial (Hartley y col., 2000) o privación del sueño, y a altas dosis puede dar lugar al síndrome denominado cafeinismo, cuyo cuadro clínico incluye ansiedad, insomnio, depresión, agitación, taquicardia e hipertensión (Nehlig y col., 1992). Por otro lado, la cafeína parece ser capaz de incrementar el riesgo de aborto espontáneo (Giannellia y col., 2003). Además, el consumo agudo de cafeína se ha asociado a un aumento del colesterol sanguíneo y parece ejercer un efecto negativo en la tolerancia a la glucosa y la sensibilidad a la insulina (Farah, 2012).

Las diversas propiedades adversas para la salud vinculadas al consumo de cafeína ha impulsado el desarrollo de diversos procedimientos para su eliminación del grano de café verde. Por ello, actualmente los productos descafeinados ocupan un lugar de importancia en el mercado de los alimentos que contienen cafeína. La cantidad de cafeína que ha de tener el café tostado para ser considerado café descafeinado varía en función de la normativa del país del que se trate. En general, en Estados Unidos el contenido de cafeína debe ser menor del 3 % (peso seco), mientras que en Europa la cantidad máxima de cafeína es 0,1 % (Clark, 2003[c]). Las principales plantas de producción de café descafeinado se encuentran mayoritariamente en Europa y Estados Unidos, donde el consumo de café descafeinado es más alto (Kumar y Ravishankar, 2009).

En el proceso de descafeinado, no sólo se busca conseguir un producto prácticamente exento de cafeína, sino también recuperar y purificar la cafeína extraída (Clark, 2003[c]) debido a su interés en diversos sectores comerciales, como por ejemplo, la industria alimentaria (bebidas energéticas y refrescos) y la industria cosmética y farmacéutica al utilizarse como principio activo (Mandel, 2002; Kumar y Ravishankar, 2009) y agente hidrotrópico (Evstigneev y col., 2006).

El descafeinado comercial se lleva a cabo en la actualidad mediante la utilización de disolventes. Si bien se han estudiado procedimientos alternativos, como la degradación de cafeína utilizando enzimas microbianas, o la producción de café modificado genéticamente que contenga un bajo contenido de cafeína, estos estudios

necesitan un desarrollo más profundo, tanto legislativo como de proceso, para ser utilizados comercialmente (Kumar y Ravishankar, 2009). Así, la eliminación de cafeína de los granos de café verde se lleva a cabo en la actualidad mediante su extracción utilizando disolventes en estado líquido o dióxido de carbono en estado supercrítico.

Producción de café descafeinado

El primer proceso de descafeinado fue el llamado "directo", utilizando disolventes orgánicos en los que la cafeína presenta una alta solubilidad, siendo el alemán Ludwig Roselius, en 1912, el primer productor comercial de café descafeinado (Ramalakshmi y Raghavan, 1999). En este procedimiento, el rendimiento de extracción de cafeína es bajo, los tiempos de extracción, largos, y se realiza un humedecimiento previo de los granos, con vapor y sumergiéndolos en agua caliente (alrededor de 60 ºC), hasta un contenido de humedad de 20-55 % en peso. De esta forma se produce, por un lado, un hinchamiento y la apertura celular de los granos que mejora la difusión de la cafeína en el disolvente y, por otro, una hidrólisis de los complejos cafeína-clorogenatos que favorecerá la liberación y la extracción de cafeína (Clark, 2003[c]; IARC, 1991). Una vez humedecido el grano, el proceso se realiza en baterías de extracción (de cinco a ocho columnas), a temperaturas de extracción cercanas a las del punto de ebullición del disolvente y durante tiempos de extracción de hasta diez horas. Finalmente, los granos se lavan y secan para evaporar el disolvente y eliminar la humedad hasta alcanzar un contenido en agua similar al inicial. La cafeína disuelta en el disolvente se recupera mediante destilación a vacío en múltiples etapas, y se purifica para ser utilizada en la elaboración de otros productos. La corriente de disolvente orgánico purificado se recircula de nuevo hacia las columnas de extracción (Farah, 2012; Clark, 2003[c]).

Se han utilizado numerosos disolventes orgánicos para este fin. El primero fue el benceno, que debido a su flamabilidad y toxicidad fue sustituido por los disolventes clorados, como el tricloruro de acetileno, cloroformo, o cloruro de metileno, siendo éste último uno de los más utilizados comercialmente. La solubilidad de cafeína en cloruro de metileno es alta (8,1 % en peso, a 25 ºC) (Shalmashi y Golmohammad, 2010), pero existen evidencias que sugieren que podría tratarse de un disolvente carcinógenico (Lynge y col., 1997; Wang y col., 2009), por lo que su uso comercial ha ido reemplazándose por el acetato de etilo, un disolvente con una toxicidad mucho menor, que aparece de forma natural en varios alimentos, por lo que el descafeinado con acetato de etilo se ha llamado "descafeinado natural". Otros disolventes utilizados han

consistido en aceites vegetales, entre ellos, el aceite de café y aceite de girasol (Hossain y Chong, 2011; Kumar y Ravishankar, 2009; Clark, 2003[c]; Ramalakshmi y Raghavan, 1999; IARC, 1991).

Otro proceso consiste en la extracción "indirecta" de cafeína en la que, a diferencia del anterior, se realiza una primera etapa de extracción con agua del grano de café verde. Posteriormente, la cafeína se extrae del agua utilizando el disolvente orgánico, quien de este modo no entra en contacto directo con el café. Sin embargo, la extracción con agua provoca la extracción de más compuestos junto con la cafeína, por lo que para prevenir su extracción en la medida de lo posible, se añade al agua una concentración determinada de sólidos solubles distintos a la cafeína. La cafeína se recupera y purifica y el disolvente libre de cafeína se recircula para volver a ser utilizado en la extracción de cafeína procedente de la fase acuosa. Los granos de café verde descafeinados se lavan con agua para eliminar solutos solubles adheridos. Por último, los granos de café descafeinado se secan hasta alcanzar su contenido de humedad inicial (Clark, 2003[c]).

El proceso indirecto es más complejo que el directo, pero es más rápido (en torno a 8 horas), se lleva a cabo un tratamiento térmico de los granos más suave, se obtienen mayores rendimientos de extracción de cafeína y una mayor pureza de la cafeína recuperada. No obstante, la menor selectividad del agua podría provocar una mayor pérdida de compuestos solubles en agua que pudieran actuar como precursores del aroma y/o sabor durante el tueste, por lo que, para compensarlo, los sólidos solubles distintos a la cafeína presentes en la corriente de agua se añaden al grano tras el proceso, consiguiéndose una reabsorción parcial de los mismos (Farah y col., 2006; Clark, 2003[c]; Ramalakshmi y Raghavan, 1999).

Otro procedimiento de descafeinado es el llamado proceso con agua Suizo (Swiss Water® Process). En este caso la eliminación de cafeína del café se lleva a cabo utilizando únicamente agua, eliminándose la etapa de extracción con disolventes orgánicos y su destilación posterior. Tras aproximadamente 8 horas de extracción, el agua cargada de cafeína se dirige a una batería de columnas de filtros de carbón activo, que retienen selectivamente las moléculas de cafeína. El carbón activo es previamente cargado con otros compuestos del café o con compuestos de una estructura y tamaño molecular similar, principalmente carbohidratos, para que así el carbón activo retenga la menor cantidad posible de compuestos distintos a la cafeína, procedentes del extracto

acuoso. Finalmente, el carbón activo se reactiva produciéndose la desorción de la cafeína (Farah, 2012; Clark, 2003[c]).

Por último, la innovación tecnológica más reciente en procesos de descafeinado consiste en la aplicación de $SCCO_2$. Se han estudiado otros gases comprimidos en la extracción de cafeína, como por ejemplo, el óxido nitroso. Sin embargo, el $SCCO_2$ es el utilizado comercialmente y el gas comprimido más estudiado en el proceso de descafeinado (Ramalakshmi y Raghavan, 1999). Este disolvente muestra una gran selectividad por la cafeína, no obstante, la solubilidad de cafeína en $SCCO_2$ es baja (Iwai y col., 2006), por lo que, para aumentar la eficacia de la extracción, es necesario que los granos de café estén hidratados.

Este proceso fue desarrollado principalmente por Zosel, quien patentó varios procesos llevados a cabo a presiones, temperaturas y tiempos entre 12-25 MPa, 40-100 ºC y 10-30 horas, respectivamente, para la obtención de un producto prácticamente exento de cafeína (cantidades menores al 0,1 % en peso). En ellos, la recuperación de cafeína se realizaba a través de una etapa de adsorción con carbón activo u otro adsorbente (como por ejemplo, gel de sílice), el cuál podía estar situado fuera o dentro del propio recipiente de extracción, o a través de una etapa de absorción de la cafeína con agua, mediante del contacto entre el $SCCO_2$ cargado de cafeína, con una fase acuosa (Zosel, 1974, 1981[a], 1981[b]). Aunque con la simple despresurización del $SCCO_2$ es posible obtener un fluido libre de cafeína que pueda ser recirculado hacia el recipiente de extracción, la ventaja de utilizar una etapa de filtrado con carbón activo o de absorción con agua es que no se necesita la despresurización del fluido supercrítico, por lo que puede recircularse hacia el extractor sin necesidad de realizar una etapa de recompresión.

Pese a ser un proceso más complejo que el llevado a cabo con disolventes líquidos y requerir equipos con un coste económico superior, el hecho de no utilizar disolventes orgánicos hace que esta tecnología esté mejor valorada. Además, la mayor selectividad del $SCCO_2$ en la extracción de cafeína genera un producto descafeinado que conserva mejor las características organolépticas del producto original (Clark, 2003[c]).

Se han desarrollado una gran cantidad de patentes en relación a los procesos de descafeinado de granos de café verde, tanto utilizando disolventes líquidos como $SCCO_2$, en las que se han estudiado numerosas variantes, tanto en el diseño del proceso,

como en los disolventes utilizados. La Tabla 1.4 muestra algunas de las patentes publicadas relacionadas con la producción de café descafeinado.

Tabla 1.4. Patentes relacionadas con el procedimiento de extracción de cafeína en la producción de café descafeinado a partir de granos de café verde.

Nombre	Disolventes	Número
Process for the production of coffee free from caffeine	Cetonas o alcoholes	US 1629512
Method for producing coffee free from caffeine	Agua con ésteres del ácido acético	US 2016634
Process for the decaffeination of raw coffee beans	Agua con ésteres de un ácido orgánico y una cetona	US 4207352
Decaffeination of green coffee with n-butyl acetate	Acetato de n-butilo	US 4562083
Coffee decaffeination with caffeic acid	Agua y precipitación de lacafeína con ácido cafeico	US 4767634
Process for the decaffeination of green coffee	$SCCO_2$	US 4728525
Supercritical carbon dioxide decaffeination of acidified coffee	Humedecimiento previo de los granos con ácido cítrico y extracción con $SCCO_2$	US 5288511
Supercritical fluid extraction method of caffeine	$SCCO_2$ con aceite de coco (triglicéridos de caprílico / cáprico)	KR 20060119032

En relación a la extracción con disolventes en estado líquido, Kündig (1924), en la patente US 2016634, llevó a cabo el descafeinado de los granos de café utilizando distintas cetonas (por ejemplo, dietilcetona) o alcoholes (por ejemplo, alcohol alílico y alcohol propílico) como disolventes de extracción, en sustitución de los disolventes clorados. Más tarde, Grethe (1935), en la patente US 2016634, utilizó agua como disolvente a la que se le añadió un éster del ácido acético, principalmente un éster etílico. En la patente US 4207352, Kurzhals (1980) intentó eliminar varios problemas derivados del uso de los anteriores disolventes, como es el elevado tiempo de secado requerido o la aparición de manchas en el grano tras el tostado al descafeinar con cetonas, así como la formación de sabores indeseables producidos al descafeinar con ésteres del ácido acético. Para ello utilizó agua a la que se añadió un éster de un ácido orgánico de bajo peso molecular (ésteres del ácido fórmico, propiónico y butírico) en combinación con una cetona de bajo punto de ebullición (acetona). En cambio, en la patente US 4562083, Gottesman (1985) propuso el uso de acetato de n-butilo de forma

directa o indirecta sobre el café, y Kaleda y col. (1988), en la patente US 4767634, añadieron ácido cafeico al disolvente de extracción (preferiblemente agua) para formar complejos insolubles con la cafeína y así purificarla.

Respecto a la utilización de fluidos supercríticos, además de los procesos desarrollados por Zosel (1974, 1981[a], 1981[b]), anteriormente comentados, Toro y Quijano (1988), en la patente US 4728525, llevaron a cabo el descafeinado de los granos de café con $SCCO_2$, pero, a diferencia de otros procedimientos, el proceso no se realizó de manera isotérmica, sino con incrementos secuenciales de la temperatura de extracción, desde 60 ºC hasta 85 ºC, con el objetivo de aumentar el rendimiento y reducir el tiempo de extracción. Más tarde, Kazlas y col. (1994), en la patente US 5288511, adicionaron previamente ácido cítrico al agua en el humedecimiento de los granos, para así compensar la disminución en el pH final de la bebida generada por la co-extracción de ácidos del café que se genera en la extracción supercrítica de cafeína. Por el contrario, Lee y col. (2006), en la patente KR 20060119032, utilizaron aceite de coco en la extracción supercrítica de cafeína, con el fin de obtener un producto descafeinado y un residuo de cafeína disuelto en aceite que pudiera ser usado como ingrediente en un producto cosmético.

En otro trabajo de extracción de cafeína con $SCCO_2$, se estudiaron distintos cosolventes. Azevedo y col., (2008) estudiaron la extracción de cafeína, aceite de café y ácidos clorogénicos de los granos de café verde, utilizando alcohol isopropílico o etanol como cosolventes (5 % en peso). En todos los casos, al aumentar la presión se produjo un aumento de la extracción de cafeína. Respecto a la temperatura, en el caso del CO_2 puro, se ha descrito un punto de inversión de la solubilidad alrededor de 20 MPa (Saldaña, 1997). En cambio, con el uso de los cosolventes, este punto de inversión no se observó y el aumento de la temperatura provocó un aumento de la cantidad de cafeína extraída a todas las presiones de estudio. No obstante, el rendimiento de extracción de cafeína obtenido con estos cosolventes fue menor que humedeciendo previamente el café y saturando con agua el $SCCO_2$ (Kopcak y Mohamed, 2005).

Se han desarrollado y aplicado numerosos procesos y disolventes con el objetivo de obtener de forma eficaz un producto descafeinado. Los disolventes clorados han demostrado ser los más selectivos, pero, como se ha mencionado anteriormente, su uso tiende a eliminarse como consecuencia de su toxicidad y de los problemas

medioambientales que generan. Por el contrario, el agua y el acetato de etilo no presentan estos inconvenientes, pero el agua es un disolvente poco selectivo y el acetato de etilo, pese a ser un disolvente más selectivo, se produce mediante síntesis química y no es de origen agroquímico. Asimismo, se han estudiado varios cosolventes en el descafeinado con fluidos supercríticos, sin obtener un aumento significativo en el rendimiento de extracción de cafeína. Por ello, el desarrollo de procesos de extracción que utilicen disolventes agroquímicos, no tóxicos y respetuosos con el medio ambiente para la extracción de cafeína de forma eficiente y selectiva, constituye un campo de estudio interesante.

1.4.2. Té verde

El té es el producto elaborado a partir de las hojas y brotes de la planta *Camellia sinensis*, (Figura 1.8) perteneciente a la familia *Theaceae*, siendo las variedades comercialmente más comunes, la sinensis (*C. sinensis* var. *sinensis*) y la assamica (C. *sinensis* var. *assamica*). La planta de té se cultiva principalmente en Asia, África y América del Sur, siendo China, India, Kenia, Sri Lanka e Indonesia los principales productores del cultivo (Wachira y col., 2013).

La infusión preparada a partir de esta planta es una de las bebidas más consumidas a nivel mundial debido a sus propiedades sensoriales y efectos estimulantes y en los últimos años ha sido intensamente estudiada por sus potenciales propiedades beneficiosas sobre la salud. Es por ello que el consumo de té en Europa y Estados Unidos haya aumentado considerablemente en los últimos 20 años (Engelhardt., 2010).

Figura 1.8. Planta de té (*Camellia sinensis*).

Actualmente, el té no solo se consume en forma de infusión, sino que también se utiliza como ingrediente en la elaboración de productos alimenticios, tales como bebidas embotelladas, productos de repostería y helados, o en productos cosméticos y fitoterápicos (Kanda y Makino, 2013; Shi y Schlegel, 2012; Kawakatsu y col., 1995).

La composición química del té y su calidad varía en función de varios parámetros, tales como la variedad genética de la planta, las condiciones climáticas, las propiedades del suelo, la posición de la hoja en el tallo (las hojas más alejadas de los brotes son las más antiguas y su composición difiere de las más jóvenes) o la época y tipo de recolección, procesado y almacenamiento de las hojas (Lee y col., 2014).

Respecto al procesado, básicamente se producen seis tipos de té a partir de la planta *Camellia sinensis*: té verde, negro, oolong, amarillo, pu-erh y blanco, siendo el té negro el más consumido a nivel mundial (alrededor del 70% de la producción mundial),

seguido del té verde (alrededor del 25%), cuya producción ha estado creciendo a un ritmo mayor que la de té negro.

Las etapas y técnicas empleadas en la elaboración de té difieren de unos tipos de té a otros. No obstante, podemos dividir las categorías de té en función del grado de oxidación al que han sido sometidas las hojas. El té verde se caracteriza por carecer de una etapa de oxidación enzimática en su procesado, a diferencia del té negro, en el que se produce una conversión completa de ciertos compuestos de la hoja, principalmente los flavonoles, por parte de las enzimas endógenas presentes (Wan y col., 2009).

En la elaboración de té verde (Figura 1.9), los brotes y hojas son recolectados manual o mecánicamente y rápidamente se someten a una etapa de desactivación enzimática mediante calor. Una vez desactivadas, las hojas de té se someten a una etapa de enrollado y finalmente a la reducción del contenido de humedad por debajo del 6 % (Wan y col., 2009).

Figura 1.9. Esquema general del procesado de la planta *Camellia sinensis* en la producción de té verde.

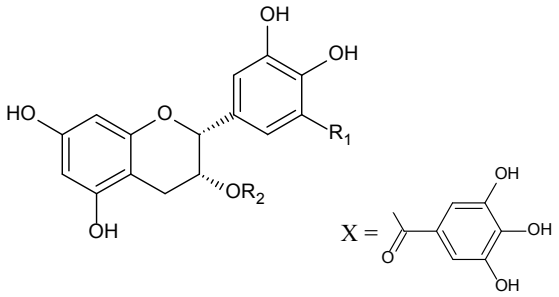
Debido principalmente a la inactivación enzimática al inicio del procesado, el té verde posee unas características organolépticas bien diferenciadas respecto a los tipos de té que han sido sometidos al proceso de oxidación (Chaturvedula y Prakash, 2011) y en cierta medida mantendrá inalterada la composición química original de la hoja de té fresca (Wan y col., 2009), presentando compuestos en mayor cantidad que en otros tipos de té, como por ejemplo ácidos orgánicos o carotenoides (principalmente luteína y β-caroteno). A diferencia de los tés fermentados, el té verde es una fuente abundante de compuestos fenólicos, que pueden representar hasta el 30 % del peso seco de la hoja de té (Shi y Schlegel, 2012; Shahidi y Naczk, 2004).

Respecto a los compuestos fenólicos, las hojas de té verde contienen principalmente flavonoles y flavonas, sobre todo en forma de glucósidos de flavonol (mono, di y triglucósidos de camferol, miricetina y quercetina) y glucósidos de flavona (mono y diglucósidos de apigenina y luteolina), así como ácidos fenólicos, siendo el más abundante el ácido 5-O-galoilquínico. Estos grupos de compuestos no se ven excesivamente alterados durante el proceso de oxidación, por lo que su concentración

en la hoja puede ser similar a la de otros tipos de té. En cambio, las proantocianidinas (entre 0,1-2 % en peso en la hoja de té verde) y, sobre todo, el grupo de compuestos fenólicos más abundante en la hoja de té, las catequinas (Engelhardt, 2010), se reducen considerablemente durante el proceso de oxidación.

Los monómeros de catequina (flavan-3-ol) son los compuestos fenólicos más abundantes de la hoja fresca de té, con un contenido en el té verde de entre el 7-25 % en peso seco, llegando en algunos casos a representar el 90 % de la masa total de compuestos fenólicos presentes (Wei y col., 2011; Engelhardt, 2010; Shahidi y Naczk, 2004). Durante el proceso de oxidación se produce la polimerización y condensación de las catequinas, originando principalmente teaflavinas y tearubiginas, por lo que el té verde contiene cantidades mayores de estos compuestos que los tés sometidos a un proceso de oxidación, más aún cuanto mayor sea la intensidad de la etapa oxidativa (Engelhardt, 2010).

Los monómeros de catequina (Figura 1.10) contribuyen, junto con otros compuestos, a la astringencia y el ligero amargor de la infusión de té, y son uno de los componentes con mayor bioactividad de las hojas de té (Chaturvedula y Prakash, 2011). Pertenecientes al grupo de los flavonoides (2-fenilcromen-4-ona), dependiendo de la configuración estereoquímica del 3′,4′-dihidroxifenil y del grupo hidroxilo en la posición 3 del anillo C, las catequinas pueden existir como dos isómeros: trans-catequinas (formas no epi) y cis-epicatequinas (formas epi). A su vez, cada uno de ellos puede aparecer como dos isómeros ópticos: (+)-catequinas / (-)-catequinas y (+)-epicatequinas / (-)-epicatequinas, respectivamente. (-)-Catequinas pueden aparecer esterificadas en la posición 3 del anillo C con una molécula de ácido gálico y originar las formas (-)-catequina-3-galato, (-)-epicatequina-3-galato, (-)-galocatequina-3-galato y (-)-epigalocatequina-3-galato, siendo la (-)-epicatequina, (-)-epigalocatequina, (-)-galato de epicatequina y (-)-galato de epigalocatequina, las principales formas presentes en la hoja de té verde. De entre ellas, el (-)-galato de epigalocatequina es, generalmente, la más abundante, seguido de la (-)-epigalocatequina y (-)-galato de epicatequina, aunque varía considerablemente en función del origen geográfico, mientras que los contenidos de (-)-epicatequina y (±)-catequina suelen ser menores (Persson, 2013; Engelhardt, 2010; Shahidi y Naczk, 2004).



R^1	R^2	Compuesto
H	H	Epicatequina
OH	H	Epigalocatequina
H	X	Galato de epicatequina
OH	X	Galato de epigalocatequina

Figura 1.10. Estructura química de los principales monómeros de catequina presentes en té verde.

Las catequinas son sensibles a la degradación, oxidación y a la epimerización, reacciones que se producen de forma simultánea como consecuencia de la presencia de oxígeno y temperaturas elevadas. Del mismo modo, el pH y el tipo de ácido presente son otros factores que afectan a la estabilidad de las catequinas, produciéndose una mayor estabilidad de las mismas a pH ácido, principalmente a valores de pH menores de 4 (Wang y col., 2008; Su y col., 2003; Wang y Helliwell, 2000; Chen y col., 1998).

Se han descrito numerosas propiedades beneficiosas para la salud derivadas del consumo de té verde. Estas propiedades han sido atribuidas principalmente a los compuestos fenólicos, en especial a las catequinas, aunque otros compuestos como carotenoides y compuestos lipídicos, como el tocoferol, podrían ejercer un efecto sinérgico (Shi y Schlegel, 2012).

Entre las propiedades beneficiosas para la salud atribuidas al consumo de catequinas destaca la actividad antioxidante (Bansal y col., 2012; Shi y Schlegel, 2012; Erba y col., 2005) y anticancerígena (piel, cavidad bucal, hígado, vejiga, pulmón, mama, tracto gastrointestinal, próstata, páncreas) (Härdtner y col., 2012; Yang y col., 2011; Chen y col., 2011; McMillan y col., 2007; Zaveri, 2006). Asimismo, parecen ejercer una acción preventiva frente a enfermedades cardiovasculares, diabetes tipo 2, obesidad y frente a ciertas enfermedades neurológicas (Khan y Mukhtar, 2007), así como una acción anti-inflamatoria (Singh y col., 2010), antiviral, antibacteriana y antifúngica (Osterburg y col., 2009; Friedman, 2007; Song y col., 2005).

Al igual que en el café, otro de los componentes con mayor bioactividad en las hojas de té verde son los alcaloides. El principal alcaloide presente en la hoja té verde es la cafeína, situándose su cantidad en torno al 2-5 % en peso seco, cuya concentración es

similar a la del té negro (Shi y Schlegel, 2012; Engelhardt., 2010). Otros dos alcaloides, también con propiedades estimulantes, pero presentes en una concentración muy baja en la hoja son la teobromina (3,7-dimetilxantina), cuya cantidad se sitúa en torno a 0,1-0,4 % en peso, y la teofilina (1,3-dimetilxantina), igualmente en pequeña concentración, inexistente en algunos casos, o no siempre detectable (Zhao y col., 2011; Engelhardt, 2010).

Producción de té descafeinado

Para tratar de evitar el uso de disolventes en la producción comercial de té descafeinado, se han estudiado varias alternativas. Una de ellas ha sido retrasar el período de recolección de las hojas y acortar el tiempo de la etapa de enrollado (Miyagishima y col., 2011). Este método presenta varios inconvenientes, ya que, pese a obtenerse una hoja con un contenido menor de cafeína, esta disminución no es suficiente para poder ser considerado té descafeinado, cuyo contenido de cafeína debería ser como máximo de 0,4 % en peso seco (Vuong y Roach, 2014). Además, un retraso en la recogida de la hoja genera una reducción de la calidad de la bebida. Otra alternativa ha consistido, al igual que en el caso del café, en el uso de microorganismos y sus enzimas (Ramarethinam y Rajalakshmi, 2004). Al tratarse de un método cuya eficacia no está comprobada y que aún requiere un mayor estudio, actualmente los dos métodos comúnmente utilizados en el descafeinado de té a nivel comercial consisten en la extracción de cafeína mediante disolventes líquidos y en la extracción con dióxido de carbono supercrítico, tal y como ocurre con el descafeinado de café.

En este caso, las hojas secas de té verde también son humedecidas para aumentar la difusión de cafeína, llevándose a cabo, en ocasiones, en presencia de amonio o sales carbónicas para provocar la ruptura de los complejos de cafeína, quedando así en su forma libre (Senol y Aydin, 2006). Al igual que con el café, se han utilizado disolventes como benceno y disolventes clorados como cloroformo, cloruro de metileno, tricloruro de acetileno, o tetracloruro de carbono, así como bases y ácidos fuertes, como el hidróxido de amonio y el ácido sulfúrico. El uso de todos estos disolventes se ha ido reduciendo debido a su toxicidad y a los problemas medioambientales que generan, por lo que el acetato de etilo es actualmente uno de los disolventes orgánicos más utilizados en el descafeinado industrial del té (Vuong y Roach, 2014).

La extracción con CO_2 supercrítico, al igual que en el café, constituye otro proceso ampliamente utilizado en la producción comercial de té descafeinado, acerca del cual se

han patentado distintos diseños en los que el CO_2 se encuentra generalmente saturado con agua cuando se pone en contacto con las hojas, a una temperatura entre 20-80 ºC y presiones de hasta 30 MPa (Lack y col., 2001; Theissing y col., 1992; Vitzthum y Hubert, 1979). No obstante, en los últimos años se ha estudiado también la extracción de cafeína utilizando como modificador etanol o sus mezclas con agua (Park y col., 2012; Sun y col., 2010).

A diferencia del café, cuya etapa de descafeinado se realiza generalmente antes del tueste, por lo que aún no se han desarrollado las características sensoriales propias del café, el descafeinado del té se realiza sobre el producto ya transformado. Esto es especialmente importante en el caso del té negro, ya que el descafeinado de las hojas se realiza tras el proceso de oxidación, por lo que, para evitar la pérdida de los compuestos volátiles, en el descafeinado se lleva a cabo una primera etapa de extracción de esos aromas, los cuales se añaden de nuevo al té una vez finalizado el proceso de extracción de la cafeína.

Pese al desarrollo de distintos métodos y al uso de numerosos disolventes, la pérdida de compuestos bioactivos (como por ejemplo, catequinas) producida durante el descafeinado parece ser inevitable (Henning y col., 2003).

Se han llevado a cabo numerosos estudios acerca de la extracción de cafeína y catequinas de hojas de té verde, y su posterior separación. En general, los trabajos publicados se podrían agrupar en tres grupos: los estudios relacionados con la producción de extractos ricos en cafeína y/o catequinas utilizando disolventes líquidos (Tabla 1.5), los trabajos relacionados con la producción de hojas de té verde con un bajo contenido en cafeína (Tabla 1.6), y por último, los estudios relacionados con la producción de extractos de té verde, ricos en catequinas y con un bajo contenido en cafeína (Tabla 1.7).

La Tabla 1.5 muestra diferentes trabajos relacionados con la extracción de cafeína y/o catequinas de hojas de té verde, en los que se estudian distintos procedimientos, condiciones de extracción y disolventes.

Tabla 1.5. Trabajos relacionados con la producción de extractos ricos en cafeína y/o catequinas de hojas de té verde utilizando disolventes líquidos.

Condiciones de extracción	Disolvente	Rendimiento de extracción	Referencia
Extracción sólido-líquido convencional			
25 °C, 20 min, 20 (m/m)[a]	acetato de etilo	Catequinas: 356,4 mg/g extracto Cafeína: 121,8 mg/g extracto	Gadkari y col. (2014)
75 °C, 30 min, 62,5 (m/m)	cloruro de colina / etilenglicol (1:5) + 30 % de agua	Catequina, ECG, EGCG: 3,6; 35,3 y 114,2 mg/g planta	Zang y col. (2014)
0,51 MPa, 20 ºC, 90 min, 19,5 (m/m)	éter dimetílico líquido	Catequinas: 5,0 mg/g extracto Cafeína:0,55 mg/g extracto	Kanda y col. (2013)
Mezcla en ebullición, 120 min, 20 (m/m)	acetona / agua (25:75)	Catequinas: 188,1 mg/g planta Cafeína: 27,2 mg/g planta	Perva-Uzunalic y col. (2006)
70 ºC, 7 min, 100 (m/m)	agua con ácido cítrico (pH 3)	Catequinas: 588,8 mg/mL extracto	Zimmermann y Gleichenhagen (2011)
80 ºC, 30 min, 20 (m/m)	agua (pH 5)	Catequinas: 88,8 mg/g planta Cafeína: 27,4 mg/g planta	Vuong y col., (2013[a])
80 ºC, 30 min, 100 (m/m)	etanol / agua (40:60)	Catequinas: 333,9 mg/g planta	Rusak y col. (2008)
25 ºC, 120 min, 200 (m/m)	etanol / agua (40:60) y 2 % ácido fosfórico	Catequinas: 131,3 mg/g planta Cafeína: 24,3 mg/g planta	Choung y Lee (2011)
Nuevas técnicas de extracción			
Ultrasonidos, 40 °C, 30 min, 10 (m/m)	etanol / agua (40:60)	Catequinas: 90,2 mg/g planta Cafeína: 19,7 mg/g planta	Choung y col. (2014)
Microondas, 90 ºC, 4 min, 20 (m/m)	etanol / agua (50:50)	Cafeína: 40 mg/g planta	Pan y col. (2003)
SWE: 3 MPa, 130 °C, 1 min, 50 (m/m)	agua	Catequinas: 3,1 mg /mL extracto Cafeína: 0,36 mg/mL extracto	Miyashita y Etoh (2013)
500 MPa, 25 ºC, 1 min, 20 (m/m)	etanol / agua (50:50)	Cafeína: 41,5 mg/g planta	Jun (2009)
500 MPa, 25 ºC, 4 min, 20 (m/m)	etanol / agua (50:50)	Catequinas: 232 mg/g extracto Cafeína: 40 mg/g extracto	Xi y col. (2014)

[a]m/m: masa disolvente / masa material vegetal

En los estudios se realizaron tanto extracciones con métodos tradicionales como extracciones con nuevas tecnologías, como la extracción asistida por ultrasonidos, o con fluidos supercríticos. Igualmente, se estudiaron distintas condiciones de extracción y distintos disolventes, siendo el agua y sus mezclas con etanol los más utilizados.

Respecto a las técnicas tradicionales de extracción, Gadkari y col. (2014) estudiaron varios disolventes (acetato de etilo, éter etílico, cloruro de metileno, cloroformo y hexano), obteniendo los extractos más puros en cafeína con los disolventes clorados, pero la mayor actividad antioxidante y contenido de catequinas con acetato de etilo y éter etílico. Zang y col. (2014) y Kanda y col. (2013) utilizaron disolventes con una toxicidad mucho menor que la de los disolventes clorados. Zang y col. (2014) estudiaron la extracción de varias catequinas con disolventes eutécticos profundos, comparándolo con agua y disolventes orgánicos (metanol, acetonitrilo, etanol y hexano), obteniéndose los mejores resultados con cloruro de colina / etilenglicol (1:5), especialmente cuando se añadió un 30 % de agua. Kanda y col. (2013) estudiaron la extracción de cafeína, catequinas y agua con éter dimetílico líquido, a partir de hojas de té verde que habían sido sometidas previamente a una infusión, con el objetivo de obtener un extracto rico en cafeína y un subproducto de té con un bajo contenido en humedad, determinándose una recuperación de catequinas de aproximadamente el 35 % y un residuo de hojas de té verde libre de cafeína. Por otro lado, Perva-Uzunalic y col. (2006) utilizaron distintos disolventes orgánicos (acetona, metanol, etanol y acetonitrilo) y sus mezclas con agua, obteniéndose las mayores recuperaciones de cafeína y catequinas con mezclas acetona / agua y acetonitrilo / agua, respectivamente. Por el contrario, Zimmermann y Gleichenhagen (2011) y Vuong y col., (2013[a]) estudiaron diferentes condiciones de extracción utilizando agua a diferentes pH. En ambos casos, la mayor concentración y rendimiento de extracción de catequinas se obtuvo a un pH menor de 5, asociado a la mayor estabilidad de los flavonoles que se produce a pH ácido. En el caso de la cafeína, el pH no pareció ejercer ninguna influencia en su extracción, a diferencia de lo descrito por Kim y col. (1999) en un estudio previo, en el que encontraron que la extracción de cafeína aumentó a un pH entre 4 y 7. Sin embargo, Rusak y col. (2008) estudiaron la extracción de estos compuestos, con agua, agua con zumo de limón exprimido y etanol acuoso a distintas concentraciones, obteniendo las mayores cantidades de catequinas con etanol acuoso,

no observando ninguna mejora respecto al agua pura al disminuir el pH como consecuencia de la adición de zumo de limón.

Respecto al uso de nuevas tecnologías de extracción, mediante extracción asistida por ultrasonidos, Choung y Lee (2011) obtuvieron cantidades similares de cafeína, pero ligeramente menores de catequinas que las obtenidas tras 24 horas de extracción a temperatura ambiente, utilizando en ambos casos mezclas etanol / agua a las que se les añadió ácido fosfórico. No obstante, el tiempo empleado en la extracción asistida por ultrasonidos fue considerablemente inferior. Más tarde, en otro estudio, Choung y col. (2014) realizaron las mismas extracciones utilizando el mismo disolvente, pero sin la adición de ácido, obteniéndose en 30 minutos de extracción asistida por ultrasonidos cantidades de catequinas ligeramente superiores a las obtenidas tras 2 horas a temperatura ambiente. Pan y col., (2003) estudiaron varios disolventes (agua, etanol, metanol, acetona) y sus mezclas con agua y varias técnicas de extracción (microondas, ultrasonidos y técnicas tradicionales de extracción), obteniendo la mayor cantidad de estos compuestos al realizar la extracción asistida por microondas con la mezcla etanol / agua. Miyashita y Etoh (2013) estudiaron la extracción de cafeína y catequinas utilizando agua como disolvente y aplicando distintas presiones y temperaturas, obteniendo extractos similares, pero en un menor tiempo de extracción cuando se utilizó la presión y temperatura más alta. En cambio, Jun (2009) estudió el efecto de las altas presiones en la extracción de cafeína, utilizando varios disolventes orgánicos (acetona, metanol, etanol) y sus mezclas con agua, obteniendo cantidades de cafeína similares a las obtenidas con procedimientos tradicionales de extracción, pero en tan solo 1 minuto de extracción. En una contribución posterior, Xi y col. (2015) determinaron la cantidad total de compuestos fenólicos extraídos a las mismas condiciones de extracción utilizadas en su anterior trabajo, obteniendo en 2 minutos de extracción cantidades muy superiores a las obtenidas a 85 ºC sin aplicar presión.

De la revisión de los trabajos publicados en la bibliografía, se concluye que, en general, el rendimiento de extracción de cafeína y catequinas es más bajo cuando se utilizan disolventes orgánicos puros, aumentando considerablemente cuando se utilizan mezclas acuosas de los mismos o bien utilizando agua a alta temperatura. Igualmente, en general, con las nuevas técnicas de extracción se obtuvieron cantidades similares o incluso mayores a las obtenidas mediante métodos tradicionales pero con tiempos de extracción considerablemente inferiores.

La Tabla 1.6 muestra los diferentes trabajos relacionados con el estudio de la producción de hojas de té verde con un bajo contenido en cafeína. En este caso, el dióxido de carbono supercrítico es el principal disolvente estudiado.

Tabla 1.6. Trabajos relacionados con la producción de hojas de té verde con un bajo contenido en cafeína.

Condiciones de extracción	Disolventes	Recuperación de cafeína y catequinas	Referencia
100 ºC, 3 min, 20 m/m[a] (material vegetal: hojas frescas)	Agua	Cafeína: 83 % Catequinas: 5 %	Liang y col. (2007)
Etapa 1: asistida con microondas, 6 min Etapa 2: 0,1 MPa, 0 °C, 150 min, 10 m/m	Agua	Cafeína: 87,6 % Catequinas: 20,1 %	Lou y col. (2012)
SFE asistida por ultrasonidos: 30 MPa, 45 ºC, 240 min, 40 % de cosolvente añadido previamente al té, 2 m/m	$SCCO_2$-agua	Cafeína: 78,4 %	Tang y col. (2010)
SFE: 30 MPa, 70 ºC, 120 min; 4,6 % cosolvente añadido previamente al té, 34 m/m	$SCCO_2$-etanol	Cafeína: 92,8 % Catequinas: 68,0 %	Park y col. (2007[a])
SFE: 30 MPa, 70 ºC, 120 min; 8,8 % de cosolvente añadido previamente al té, 34 m/m	$SCCO_2$-agua	Cafeína: 77,4 % Catequinas: 75,3 %	Park y col. (2007[b])
SFE: 40 MPa, 40 ºC, 300 min, 7 % de cosolvente añadido previamente al té; 28,1 m/m	$SCCO_2$-agua	Cafeína: 54 % EGCG: 21 %	Kim y col. (2008)
SFE: 30 MPa, 80 ºC, 120 min; 3,5 % de cosolvente, 18 m/m	$SCCO_2$-etanol	Cafeína: 70,2 % Catequinas: 6,2 %	Sun y col. (2010)
SFE: 30 MPa, 80 ºC, 450 min; 0,03 % de cosolvente añadido previamente al té, 900 m/m	$SCCO_2$-agua	Cafeína: 95,6 % Catequinas: 19,8 %	Huang y col. (2007)

[a] m/m: masa de disolvente / masa material vegetal

Liang y col. (2007) estudiaron el proceso de descafeinado de té verde utilizando agua. Los autores obtuvieron una extracción muy selectiva de la cafeína cuando el proceso se realizó sobre hojas de té verde frescas. En cambio, cuando el proceso se realizó sobre las hojas secas (tal y como se realiza comercialmente), la recuperación de catequinas fue alta (49-68 %). Lou y col. (2012) estudiaron el descafeinado de té verde mediante dos etapas de extracción: un pretratamiento con microondas durante 6 minutos, seguido de una extracción a vacío con agua a aproximadamente 0 ºC. De este modo, se extrajo el 87,6 % y se obtuvo una recuperación de catequinas y compuestos fenólicos de 20,1 % y 36,2 %, respectivamente.

Respecto al uso de fluidos supercríticos, Tang y col. (2010) llevaron a cabo la extracción de cafeína utilizando agua como cosolvente, la cual se combinó con ultrasonidos. Mediante este proceso combinado, los autores alcanzaron una recuperación de cafeína de casi el 79 %. En la mayor parte de los estudios publicados relacionados con el uso de $SCCO_2$, el cosolvente utilizado fue agua o etanol y se puso previamente en contacto con las hojas de té, humedeciéndolas antes de iniciarse el proceso con CO_2, de forma muy similar a lo que se realiza a nivel comercial. Por ejemplo, Park y col. (2007[a], 2012) estudiaron la extracción de cafeína utilizando etanol como cosolvente. A presiones entre 23 MPa y 30 MPa y temperaturas entre 60 ºC y 70 ºC, los autores obtuvieron recuperaciones de cafeína de entre el 93-97 %, pero también se produjo la extracción de otros compuestos como catequinas (recuperación entre 41-68 %) y clorofila (recuperación del 43 %). En otro trabajo, Park y col. (2007[b]) utilizaron agua como cosolvente. En este caso, a las mismas condiciones de presión y temperatura utilizadas con etanol, pero añadiendo una cantidad de cosolvente casi dos veces mayor, los autores obtuvieron una recuperación de cafeína de tan sólo el 77 %, mientras que la recuperación de catequinas (en torno a 75 %) fue más alta que con etanol. Kim y col. (2008) también utilizaron agua como cosolvente, humedeciendo previamente las hojas de té verde. En este caso, la mejor relación cafeína / EGCG obtenida fue 2,57; pero la recuperación de cafeína alcanzada fue pequeña (54 %).

Sun y col. (2010), utilizaron agua, etanol y sus mezclas, pero en este caso, las hojas de té no fueron humedecidas previamente y el cosolvente fue bombeado y mezclado previamente con el CO_2. Los mejores resultados se obtuvieron con etanol, alcanzándose una recuperación de cafeína del 70,2 % y una relación cafeína / catequinas de 11,3. Por último, Huang y col. (2007) realizaron la extracción supercrítica humedeciendo previamente las hojas de té y también, humedeciendo las hojas y adicionando seguidamente en forma continua una mezcla de CO_2 y agua. Según los resultados obtenidos, la mejor relación cafeína / catequinas se logró cuando la muestra fue únicamente humedecida con el cosolvente y no se produjo la adición posterior al CO_2.

De los trabajos publicados vinculados a la tecnología supercrítica, se concluye que combinando CO_2 con agua o etanol como cosolvente es posible alcanzar altas tasas de extracción de cafeína, pero se produce también una considerable pérdida de catequinas. Esta observación es también válida en el caso de disolventes líquidos. Tan solo Liang y col. (2007) alcanzaron altas tasas de eliminación de cafeína preservando casi por

completo el contenido de catequinas (ver Tabla 1.6), pero, como se ha comentado, estos resultados se obtuvieron a partir de hojas de té verde frescas.

La Tabla 1.7 muestra diferentes procedimientos publicados en la bibliografía para la producción de extractos de té verde descafeinado y concentrados en catequinas. En general, para obtener estos extractos los autores partieron de extractos acuosos de té verde.

Tabla 1.7. Trabajos relacionados con la producción de extractos de té verde concentrados en catequinas y con un bajo contenido en cafeína.

Método de producción	Referencia
Disolventes líquidos	
Etapa 1: Extracción de cafeína con cloroformo Etapa 2: Purificación de catequinas de la fase acuosa con acetato de etilo	Row y Jin (2006)
Etapa 1: Extracción de catequinas con acetato de etilo Etapa 2: Evaporación del extracto y disolución en agua Etapa 3: Purificación de catequinas extrayendo cafeína con cloruro de metileno	Choung y col. (2014)
Etapa 1: Extracción de catequinas con acetato de etilo Etapa 2: Purificación de catequinas precipitando cafeína con disolución de ácido cítrico	Dong y col. (2011[a])
Etapa1: Descafeinado de las hojas de té: agua, 100 ºC, 4 min, 20 m/m[a] Etapa 2: Extracción con agua (2 extracciones: 80 ºC, 30 min cada extracción, 12 m/m y 8 m/m, respectivamente) seguido de secado por atomización	Vuong y col. (2013[b])
Fluidos supercríticos	
Etapa 1: Extracción asistida por microondas Etapa 2: Precipitación SAS: 9 MPa, 15 ºC, relación CO_2 / extracto ≈ 1	Sosa y col. (2011)
Tecnología de membranas	
Nanofiltración: membrana G-10 y G-20, 5 MPa, 23 ºC, etanol / agua (80:20)	Nwuha (2000)
Formación de crema	
Hidroxipropilmetilcelulosa junto con polivinilpirrolidona	Monsanto y col. (2014)
Procesos de adsorción (adsorbente)	
Lignocelulosa (madera de cedro)	Sakanaka (2003)
Lignocelulosa (tallos de té)	Ye y col. (2009)
Lignocelulosa (madera de abeto) copolimerizada con N-vinilpirrolidona	Ye y col. (2010)
Carbón activo	Ye y col. (2007)
Poliamida-6	Ye y col. (2011)
Poli(acrilamida-co-dimetilacrilato de etilenglicol)	Lu y col. (2010)
Polivinilpolipirrolidona	Dong y col. (2011[b])
Polivinilpolipirrolidona	Fan y col. (2014)
Combinación de la tecnología de membranas y adsorción	
Ultrafiltración: membrana de acetato de celulosa - titanio y resina de poliamida	Li y col. (2005)

[a] m/m: masa de disolvente / masa material vegetal

Row y Jin (2006) llevaron a cabo la extracción de cafeína mediante dos extracciones secuenciales con cloroformo para, finalmente, extraer las catequinas de la fase acuosa con acetato de etilo. En cambio, Choung y col. (2014) realizaron una primera etapa de fraccionamiento mediante tres extracciones secuenciales con acetato de etilo, en la que más del 95 % de las catequinas presentes en el extracto inicial se recuperaron. Seguidamente, se evaporó el disolvente y el extracto seco se disolvió en agua y fue sometido a varias extracciones secuenciales con cloruro de metileno para eliminar la cafeína restante. Así, el 96,2 % de la cafeína inicial fue eliminada y sólo el 1,2 % de las catequinas se extrajeron con cloruro de metileno, demostrándose que los disolventes clorados son altamente selectivos para la cafeína. Sin embargo, como ya se ha comentado, estos disolventes tienen el inconveniente de presentar una alta toxicidad y no ser respetuosos con el medio ambiente.

Dong y col. (2011) estudiaron tres disolventes orgánicos (acetato de etilo, n-butanol y n-hexano) para aislar las catequinas, siendo el acetato de etilo el más eficaz para tal fin y, posteriormente, la cafeína fue extraída del extracto rico en catequinas mediante precipitación secuencial con una solución de ácido cítrico. En este caso, la eliminación de cafeína alcanzada fue del 78,8 %, por lo que la eficacia fue considerablemente menor que la de los disolventes clorados. Por otro lado, Vuong y col. (2013) aprovecharon la extracción selectiva de cafeína a partir de hojas frescas (Liang y col., 2007), para después obtener un extracto acuoso rico en catequinas y con un bajo contenido en cafeína, y someterle finalmente a un secado por atomización o liofilización. Pese a obtenerse un extracto en polvo con una concentración de cafeína y catequinas de 0,73 % y 19,6 % en peso, respectivamente, este método presenta el inconveniente de tener que llevar a cabo el descafeinado sobre las hojas de té frescas, ya que al realizarse sobre la hoja seca, la pérdida de catequinas sería muy elevada.

Por otro lado, Sosa y col. (2011) llevaron a cabo la precipitación y encapsulación de los polifenoles de té mediante precipitación solvente-antisolvente con $SCCO_2$, a partir de un extracto de té verde obtenido con acetona mediante extracción asistida por microondas. Pese a que el objetivo principal del trabajo consistió en el estudio de las mejores condiciones para obtener un alto rendimiento de precipitación y encapsulación de los polifenoles y catequinas, la precipitación con $SCCO_2$ permitió obtener un fraccionamiento parcial del extracto de té, ya que solo el 13 % de la cantidad de cafeína presente en el extracto fue encapsulada en el producto final.

Monsanto y col. (2014) estudiaron la separación de cafeína y catequinas mediante el efecto de formación de crema. En este caso, los autores estudiaron el efecto de tres sales y cuatro agentes precipitantes, a distintas temperaturas y pH, para maximizar la cantidad de catequinas y minimizar la de cafeína obtenidas en el precipitado. Los mejores resultados se obtuvieron utilizando los precipitantes hidroxipropilmetilcelulosa y polivinilpirrolidona. Sin embargo, pese a que la relación catequinas / cafeína se incrementó en el precipitado, también se obtuvo un 40 % de la cafeína presente en el extracto original.

Respecto a los procesos de separación con adsorbentes, se han estudiado distintos materiales para tal fin, siendo las mezclas etanol / agua las más utilizadas para llevar a cabo las etapas de desorción. En general, estos adsorbentes presentan una alta selectividad en la separación de cafeína y catequinas. Por ejemplo, Lu y col. (2010) obtuvieron una relación catequinas / cafeína de 430 partiendo de una relación inicial en el extracto de 6,94 cuando utilizaron como adsorbente la poliacrilamida entrecruzada con dimetilacrilato de etilenglicol y Li y col. (2005) realizaron un proceso combinado de ultrafiltración, seguido de adsorción con una resina de poliamida, obteniendo tras la etapa de adsorción una concentración de polifenoles de más del 90 % en peso, con una concentración de cafeína menor al 4 % en peso.

En resumen, los disolventes clorados mostraron una alta eficacia en la separación de la cafeína respecto a las catequinas. Sin embargo, presentan el inconveniente de su alta toxicidad y de no ser ecológicos. La separación de estos compuestos mediante el efecto de formación de crema no presenta esos inconvenientes, pero es una técnica poco selectiva. La tecnología de membranas presenta problemas tecnológicos como es la disminución de flujo que se produce a medida que el proceso avanza. Además, la limpieza y acondicionamiento de las membranas requiere generalmente el uso de disolventes tóxicos, tales como ácidos y bases fuertes (HCl y NaOH). Por el contrario, ciertos adsorbentes sí muestran una alta selectividad a la hora de fraccionar estos compuestos. Sin embargo, estos resultados se obtuvieron tras largos tiempos de contacto entre el adsorbente y los compuestos y/o utilizando bajos flujos en la etapa de elución. Además, en las etapas de elución se requieren grandes volúmenes de disolvente que posteriormente han de ser tratados y al igual que ocurre en la separación con membranas, los adsorbentes pierden eficacia y deben regenerarse tras un cierto número de procesos.

1.5. *Ethyl lactate: a biorenewable agrochemical solvent for food technology*

20.4 ETHYL LACTATE: A BIORENEWABLE AGROCHEMICAL SOLVENT FOR FOOD TECHNOLOGY

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20.4.1 THE GREEN SOLVENT ETHYL LACTATE

20.4.1.1 Introduction

In recent years, there has been a growing interest to replace petroleum-derived feedstocks due to several factors such as the steady increase of oil price, the more severe environmental regulations and the increasing social awareness about ecological issues. For this reason, there is a growing need for more environmentally acceptable processes in the chemical industry. This trend towards what has become known as "Green Chemistry" seeks the implementation of profitable processes and, at the same time, assigns economic value to waste materials and avoiding the use of toxic and/or hazardous substances in the production and application of chemical products. In this sense, solvents are one of the targets of Green Chemistry research, due to their comprehensive use and the large amount of waste that their use generates. Moreover, many conventional solvents are toxic, flammable, and/or corrosive. For this reason, there is a clear global trend to replace these conventional solvents by non-toxic and environmentally friendly solvents or "Green solvents", such as lower alcohols or esters.[1,2]

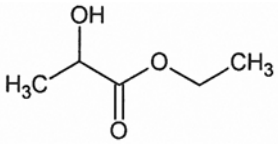


Figure 20.4.1 Molecular structure of ethyl lactate.

Green solvents are produced from the processing of agricultural crops. In this sense, lactate esters (primarily ethyl lactate) are candidates to replace many petroleum-derived and chlorinated solvents.[3] Ethyl lactate (ethyl 2-hydroxypropanoate; lactic acid ethyl ester; 2-hydroxypropanoic acid ethyl ester; actylol), is a hydroxyl ester whose molecular formula is $C_5H_{10}O_3$ (Figure 20.4.1) and can be either in levo (S) or dextro (R) form. Ethyl lactate is an agrochemical "green solvent" derived from processing of carbohydrate feedstocks, such as wastes from corn or sugar crops and is found naturally in small quantities in a wide variety of foods such as meat, some fruits, soy products and fermented foods such as wine or beer, acting as flavoring compound. Ethyl lactate is a viable alternative to traditional liquid solvents since it is fully biodegradable, non-corrosive, non-carcinogenic, non-teratogenic and non-ozone depleting. It was affirmed as GRAS (generally recognized as safe) and due to its low toxicity, was approved by the U.S. Food and Drug Administration (FDA) as pharmaceutical and food additive. Ethyl lactate has a high boiling point, low vapor pressure, low surface tension and high solvent power since ethyl lactate can cover a wide range of polarities.[4] All these features have increased the attention to the use of ethyl lactate as a green solvent for many industrial applications, replacing traditional toxic halogenated and petroleum-based solvents.

Ethyl lactate is produced by esterification of lactic acid with ethanol and this process can be done in biorefineries. In these plants, biomass as raw material is transformed into biobased products such as proteins, acids, alcohols, fibers or energy (biogas).[5] In the case of ethyl lactate, both ethanol and lactic acid can be produced in biorefineries from a variety of biomass crops, such as sugar, starch or cellulosic feedstocks, particularly from wastes,[6] resulting also in an economic revaluation for by-products.

20.4.1.2 Physical and thermodynamic properties

Aparicio and Alcalde[7] carried out a theoretical study on S-ethyl lactate, the most stable enantiomer. The molecular structure of ethyl lactate is mainly defined by its capacity to develop intermolecular hydrogen bonding and, due to the relative position of the hydroxyl hydrogen with regard to the oxygen, its ability to form strong intramolecular hydrogen bonding between the hydroxyl hydrogen and carbonyl oxygen and/or alkoxy oxygen (intramolecular hydrogen bonds occur principally among hydroxyl and carbonyl groups rather than hydroxyl and alkoxy groups).

Furthermore, the effect of the surrounding media on intramolecular hydrogen bonding was studied. In this regard, the development of intramolecular hydrogen bonding is weakened when the relative permittivity of the surrounding medium increases.[8] Aparicio *et al.*[9] have reported an ethyl lactate relative permittivity value of 15.7 at 298.15K, so it is a moderately polar solvent, and have concluded that ethyl lactate develops preferably intermolecular hydrogen bonding with neighboring ethyl lactate molecules instead of intramolecular hydrogen bonding, when ethyl lactate is mixed with a fluid having polarity comparable to that of ethyl lactate.

At both gas and liquid phase, the formation of multimers by ethyl lactate has been described.[7,10] These associations can be formed by 2, 3 and 4 molecules of ethyl lactate and have different topologies and different hydrogen bonding strengths. Several dimers with different morphologies have been analyzed for ethyl lactate. The most stable dimer consists of symmetric dimer. In this case, the two ethyl lactate molecules are assembled by two reciprocal O–H$\cdots$C=O interactions with the same length, leading to an eight-membered ring structure.

With respect to experimental ethyl lactate physical properties, liquid density, refraction index and viscosity data have been measured and reported by several authors. Table 20.4.1 shows experimental ethyl lactate density values measured at atmospheric pressure and different temperatures. Table 20.4.2 presents the experimental refraction index for ethyl lactate as a function of temperature.

Table 20.4.1. Experimental ethyl lactate density at different temperatures and atmospheric pressure.

Ref.	Density (g/cm^3) @ different temperatures (K)							
	278.15	288.15	293.15	298.15	308.15	318.15	328.15	338.15
9	1.050007	1.03927		1.02838	1.01742	1.00637	0.99523	0.98396
11				1.0289	1.0187	1.0075		

Table 20.4.1. Experimental ethyl lactate density at different temperatures and atmospheric pressure.

Ref.	Density (g/cm^3) @ different temperatures (K)							
	278.15	**288.15**	**293.15**	**298.15**	**308.15**	**318.15**	**328.15**	**338.15**
12				1.0272				
13			1.032939					
14				1.02802				

Table 20.4.2. Experimental refraction index (n_D) of ethyl lactate.

Ref.	n_D @ different temperatures (K)							
	278.15	**288.15**	**293.15**	**298.15**	**308.15**	**318.15**	**328.15**	**338.15**
9	1.41973	1.41515		1.41046	1.40604	1.40142	1.39654	1.39194
12			1.4124					
13			1.4129					
14				1.41050				

In addition, Aparicio and Alcalde[7] reported ethyl lactate density and dynamic viscosity for a wide range of pressures and temperatures. Density values are relatively large, pointing to an efficient packing of the ethyl lactate molecules. Also Chen and Chu[11] and Riddick *et al.*[12] reported the dynamic viscosity for ethyl lactate at atmospheric pressure (Table 20.4.3). Ethyl lactate does not have high viscosity which could hinder its application as solvent in heat and mass transfer operations.

Table 20.4.3. Experimental dynamic viscosity (η) of ethyl lactate at atmospheric pressure.

Reference	η (mPas) @ different temperatures (K)		
	298.15	**308.15**	**318.15**
11	2.398	1.863	1.494
12	2.440		

Other physical and thermodynamic properties of ethyl lactate, such as molar volume, critical temperature, critical pressure, critical volume, heat of vaporization, and liquid heat capacity, among others, can be found in the work reported by Pereira and coworkers.[6]

20.4.1.2.1 Ethyl lactate vapor pressure

Riddick *et al.*[12], Peña-Tejedor *et al.*[13] and Resa *et al.*[14] measured the normal boiling point (ambient pressure) of ethyl lactate, determining values of 427.7, 427.65, and 424.98K,

respectively. Snell and Snell[15] determined the boiling point of 303.15K at 5 mm Hg of pressure.

Ínal[16] reported the experimental vapor pressure curve of ethyl lactate for pressures between 12-74 mm Hg and temperatures between 54-86°C (Figure 20.4.2). As can be observed, for ethyl lactate, the increase in pressure with temperature is very sharp. This result suggests a very high heat of vaporization value. Table 20.4.4 shows the Antoine constants for obtaining the vapor pressures of the ethyl lactate at different temperatures, as reported by Riddick *et al.*[12]

Villanueva *et al.*[17] correlated the ethyl lactate vapor pressure with temperature using the Group Contribution Equation of State (GC-EoS) model. A complete explanation of the GC-EoS equation and fundamentals is given by Skjold-Jørgensen.[18] In summary, this thermodynamic model has two contributions to the residual Helmholtz energy of the system: a repulsive hard sphere Carnahan-Starling type term and an attractive term, which combines the group contribution approach with the local-composition mixing rules. The pure group energy parameters together with the group energy interaction parameters required for modeling the ethyl lactate vapor pressures are given in Appendix A.

Table 20.4.4. Ethyl lactate Antoine constants.[12]

	Antoine equation $\log[P(kPa)] = A - B/[T(^oC) + C$
A	7.8269
B	2489.7
C	273.15

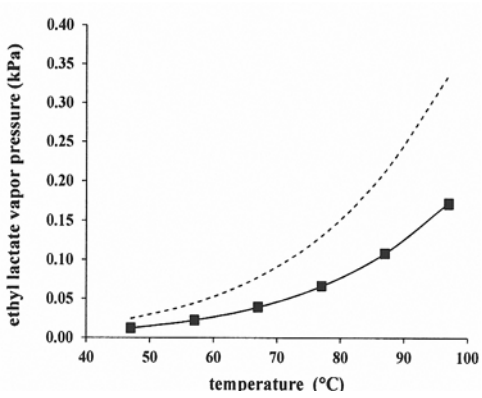


Figure 20.4.2. Experimental and calculated ethyl lactate vapor pressures: (■) experimental data;[16] (- - -) GC-EoS using Set 1 of functional groups; (——) GC-EoS using Set 2 of functional groups.

Figure 20.4.2 shows the calculation of ethyl lactate vapor pressure carried out by Villanueva *et al.*[17] as a function of temperature in the range T = (47-97)°C. According to the customary GC-EoS parameter table,[19] the chemical structure of ethyl lactate can be represented by means of two CH_3, one $COOCH_2$ and one OHCH groups (Set 1). Taking into account that ethyl lactate is the ester of lactic acid, the inclusion of an alcohol-ester functional group (CHOHCOO) seems to be more appropriate to define its chemical group composition (Set 2), as can be observed in Figure 20.4.1.1. The two different sets utilized by Villanueva *et al.*[17] to define the group composition of ethyl lactate are summarized in Table 20.4.5.

Using the GC-EoS model and Sets 1 and 2 of functional groups the ethyl lactate vapor pressure was predicted and compared with the experimental data reported by Ínal.[16] The absolute average deviations:

$$AAD = \frac{1}{N}\sum\left(\frac{p_{exp}^{vap} - p_{cal}^{vap}}{p_{exp}^{vap}}\right) \qquad [20.4.1]$$

obtained were: 96.7% and 0.39% respectively for Set 1 and Set 2. That is, the original ester group ($COOCH_2$) defined in the GC-EoS model cannot provide an accurate representation of ethyl lactate vapor pressure. On the contrary, using the ester-alcohol group (CHOHCOO) an excellent representation can be achieved.

Table 20.4.5. Different alternatives for the definition of the group composition of ethyl lactate molecule.

Chemical group	Set 1	Set 2
$-CH_3$	2	2
$-CH_2-$		1
$-C(O)OCH_2-$	1	
$-CH(OH)-$	1	
$-CH(OH)C(O)O-$		1

These results show the useful application of group contribution thermodynamic models to predict phase equilibria properties, and the necessity of a rational partition of a molecule into appropriate functional groups that take into account the basis of its chemical bonding.

20.4.1.3 Commercial production of ethyl lactate

Ethyl lactate is commercially produced by esterification of lactic acid with ethanol with water as by-product (Figure 20.4.3). This esterification process is important not only to produce the solvent, but also as a step in the purification of lactic acid itself. Lactic acid formed *via* fermentation from agroindustrial by-products needs purification since the fermented broth contains residual sugar compounds, organic acids, and other impurities, together with materials added during the production process such as calcium carbonate to neutralize the lactic acid.[20] In this regard, lactic acid molecules are reacted with ethanol and other alcohols such as methanol and butanol to form lactic acid esters and, once they

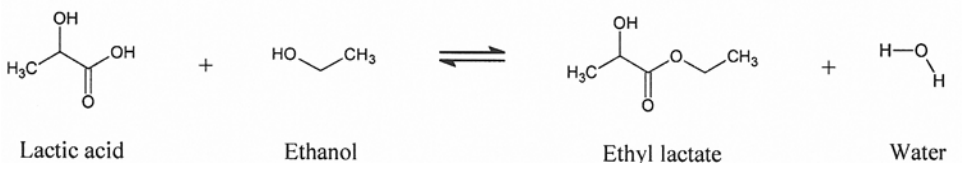


Figure 20.4.3. Esterification of lactic acid with ethanol.

have been purified, lactate esters are hydrolyzed into lactic acid and a high-purity product can be obtained.[21-24]

Ethyl lactate is also produced from ammonium lactate instead of lactic acid (Figure 20.4.4). Fermentation in the presence of ammonia to produce ammonium lactate and the reaction of this ammonium lactate with ethanol gives ethyl lactate and ammonia as products.[25-27] By using ammonium lactate as a feedstock for producing ethyl lactate, some post-treatment steps can be avoided, such as the addition of calcium carbonate (used to neutralize the lactic acid during fermentation) and the subsequent acidification with sulfuric acid to convert the salt into lactic acid and insoluble calcium sulfate, preventing the gypsum-generating process. Furthermore, ammonium lactate is more water soluble than calcium lactate,[28] but the esterification rate with ethanol is low and relatively large amounts of ammonium lactate are converted to lactamide, an undesired by-product if ammonia produced by dissociation of ammonium lactate is not removed rapidly.[29] In this way, Kasinathan *et al*.[30] developed a process in which ammonium lactate was fragmented into ammonia and lactic acid at high temperature in an organic solvent. Ammonia was removed in order to prevent the lactamide production, lactic acid was dissolved in the organic solvent and then lactic acid reacted with ethanol to produce ethyl lactate. Tributyl phosphate, triethyl phosphate, dimethyl sulfoxide and N-methyl pyrrolidine were assessed as solvents. Triethyl phosphate showed to be the most efficient solvent owing to it was reached higher ethyl lactate yield and lower by-products levels.

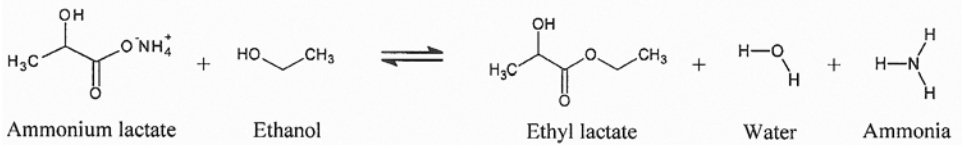


Figure 20.4.4. Esterification of ammonium lactate with ethanol.

Ethyl lactate can also be produced by using lactide (a cyclic dimer of lactic acid[31]) and polylactides as starting materials, but such processes are not economically viable since they are more expensive than the final product. Ethyl lactate itself is used to produce lactide and polylactides because ethyl lactate has a lower added value.

Lactic acid has a carboxyl and hydroxyl functional group and because of its bifunctional nature, lactic acid can undergo intermolecular esterification to form linear dimers, trimers and higher lactic acid oligomers. At the same time, these polymers can be hydrolyzed into lactic acid monomers when water is present or can undergo esterification and transesterification (alcoholysis) with ethanol, leading to a mixture of acid and ester monomer and oligomers (Figure 20.4.5). Ethyl lactate yield and the process layout are adversely affected by lactic acid oligomers formation. It has been shown that dilute lactic acid solutions are almost exclusively formed by lactic acid monomers, and at higher lactic acid concentration, lower water and ethanol concentration, and higher temperature, oligomerization rises.[32-34] However, concentrated lactic acid solutions are required in order to produce ethyl lactate economically. A certain amount of water might be beneficial to avoid

oligomerization,[35] an excess of water limits the esterification process and large quantities of alcohol and energy will be required.[36]

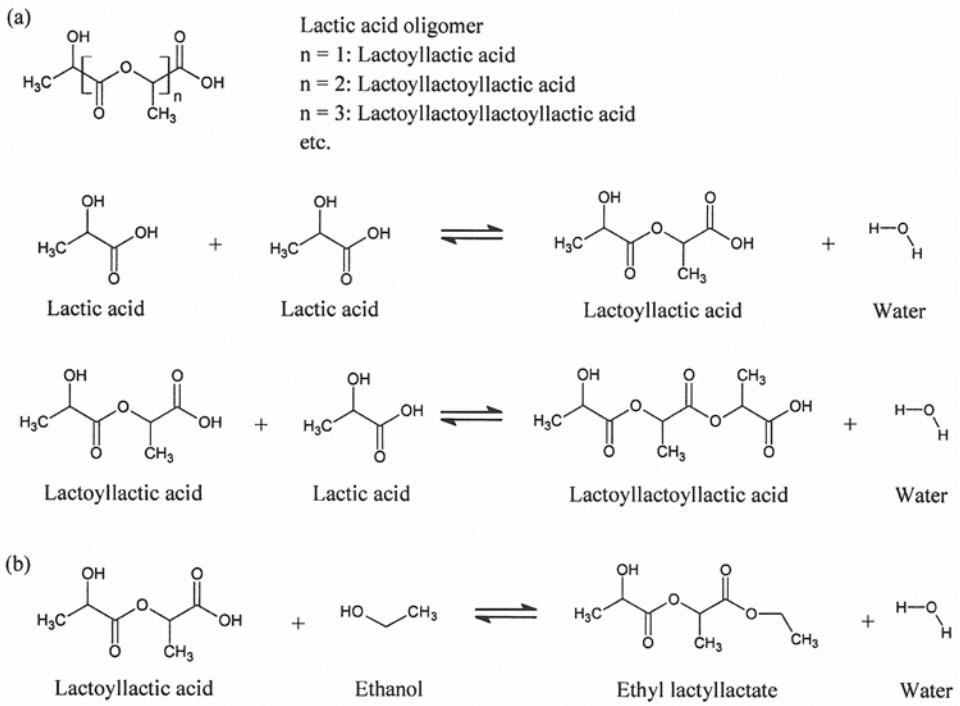


Figure 20.4.5. Lactic acid oligomerization (a), esterification of lactic acid oligomer (b) and hydrolysis equilibrium.

Esterification is a self-catalyzed reaction, which is thermodynamically limited, thereby, lower conversions and low purity products are achieved. For this reason the use of catalysts is critical. Homogeneous mineral acids, such as sulfuric acid, phosphoric acid, or hydrogen chloride, have traditionally been used to promote efficiently the esterification of varied carboxylic acids, however, their use presents drawbacks, such as the production of corrosive liquid waste, corrosion of equipment, difficulties in recovering the catalyst, or a larger amount of side reactions. Compared with this, the use of heterogeneous acid catalyst, such as acid-treated clays, heteropolyacids, zeolites, ion-exchange resins (the most commonly used solid catalysts for this purpose) like Amberlyst 15-wet, Nafion NR50, among others, which have an ecofriendly nature, provides a longer lifetime and alternative to drawbacks described above.[6]

Several authors have reported kinetic studies on the esterification of lactic acid with ethanol, carried out with different catalysts (mainly heterogeneous catalysis), at different temperatures, lactic acid/ethanol ratio and different concentrations of aqueous lactic acid.[21,34,37-41] In most studies, the presence of oligomers has not been considered, since their amount is irrelevant at equilibrium.[6]

There are a considerable number of processes in which ethyl lactate is produced by esterification until a certain amount of water is formed, equilibrium is reached and then ethyl lactate is purified by distillation or other methods. These processes require excess of ethanol to overcome the equilibrium limitations, achieve higher conversions, and, besides, the separation of the products from the equilibrium mixture is technically difficult, because of mixture of products and unconverted reactants. These are high cost operations.[6] In order to improve ethyl lactate production, an alternative to conventional method consist of combining a separation unit with reaction stage. In the hybrid processes, at least one of the products is continuously removed from the reaction medium so equilibrium is shifted to products formation according to Le Châtelier's principle. In this regard, some reactive separation processes studied for ethyl lactate production are presented below.

One of them employs membrane-based separation processes connected to the esterification reaction. In this respect, vapor permeation and pervaporation process have been tested and three different layouts have been reported for ethyl lactate production.[6] In one of them, membrane module is located outside the reactor unit and the retenate is recirculated to the reactor.[42,43] In another scheme, the membrane module is placed inside the reactor, but the membrane does not participate in the reaction directly and simply acts as a filter,[44,45] and in the third configuration, membrane itself participates in the reaction catalysis (catalytic membrane reactor).[46] Different hydrophilic membranes, such as polymeric, ceramic, zeolites and organic-inorganic hybrid membranes were tested.[6]

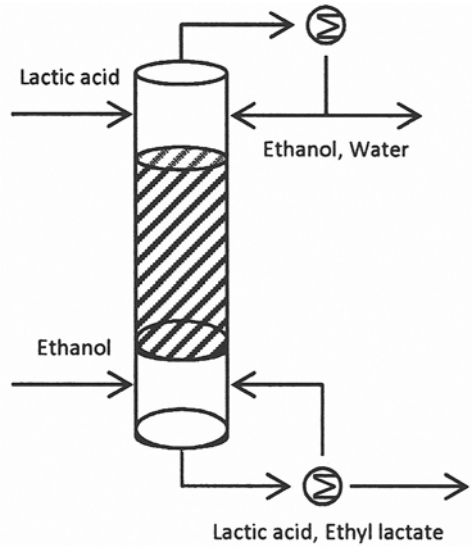


Figure 20.4.6. Basic schematic representation of a reactive distillation unit.

Another reactive separation processes studied is the reactive distillation (see Figure 20.4.6). This concept is based on an integration of an esterification reactor and a reactive distillation column in the same unit. Gao *et al.*[47] studied the effect of several operation parameters on ethyl lactate yield: flow rate of feed lactic acid, ethanol/lactic acid ratio and the effect of different feed points through the column. At studied conditions, only 52% of ethyl lactate yield was achieved. On the other hand, Asthana *et al.*,[36] using concentrated lactic acid solutions (88 wt%), achieved a lactic acid conversion of 93% and an ethyl lactate yield of 70%, with an ethyl lactate purity of 71% (mol%), avoiding the presence of water in the column bottom stream.

A novel reactive separation method consists of using simulated moving bed reactor (SMBR), a type of continuous chromatographic reactor. Pereira *et al.*[48] reported an experimentally ethyl lactate productivity of 3.36 kg_{EL}/L_{resin}·day (mass of ethyl lactate produced per unit volume of catalyst employed and unit time) and ethyl lactate purity of 75%.

The catalyst used (Amberlyst 15 – wet resin) acted also as water-absorbing resin. Experimental results were well predicted by a mathematical model developed by the authors, leading to an ethyl lactate productivity value of 31.7 kg_{EL}/L_{resin}·day, 95% of purity and a lactic acid conversion of 100%, under optimal conditions.

In order to reduce the high ethanol consumption, Silva *et al.*[49] applied the simulated moving bed membrane reactor (PermSMBR) for the production of ethyl lactate. In PermSMBR, each column contains the catalyst and a set of hydrophilic membranes to increase the water removal, leading to a better process performance. A theoretical model was developed to evaluate the PermSMBR process performance for different conditions. Authors concluded that ethanol consumption with SMBR is 152% higher than the one with PermSMBR, and 165% higher than the one with reactive distillation for a similar ethyl lactate production.

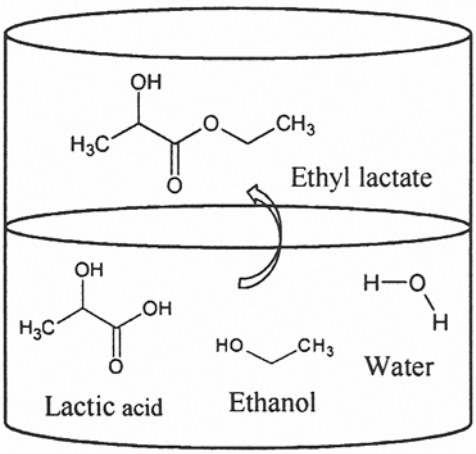


Figure 20.4.7. Scheme of the catalytic extractive reaction

Another reactive separation processes studied for ethyl lactate production is the catalytic extractive reaction (Figure 20.4.7). In this case, the esterification is performed in a biphasic liquid solvent system composed by a reactive polar liquid phase which contains the esterification constituents: lactic acid, ethanol and catalyst, and an extractive organic solvent selective of the ester. Therefore, ethyl lactate should preferably be dissolved in the extractive organic phase shifting, in this way, the reaction equilibrium to ester formation. The immiscible extractive solvent is an aromatic or other solvent like toluene, benzene or diethyl ether, among others.[50] Nevertheless, it has also been used an immiscible solvent based on fatty acid methyl ester,[51,52] but in this case, the procedure represents a method to produce an organic biosolvent and not just ethyl lactate as solvent.

Ethyl lactate production has also been studied through the enzymatic esterification of lactic acid with ethanol in ionic liquids (a non-conventional solvent), taking advantage of the enantioselectivity, regioselectivity, and chemoselectivity of enzymes such as lipase B from *Candida antárctica* (Novozyme 435), to produce enantiomerically pure ethyl lactate. Findrik *et al.*[53] using an incubator working in batch mode and using Cyphos 104 as ionic liquid, 50°C of reaction temperature, ethanol/lactic acid ratio around 3, and 8% of water content in the lactic acid, obtained 60% lactic acid conversion after 5 hours and near to 100% after 24 hours, with the water content and temperature being the most influential parameters of the variables studied. Authors also concluded that in the investigated variable range at water content (8%), ethanol/lactic acid around 11, and temperature of 30°C, maximum enantiomeric excess of ethyl lactate achieved was 34.3%, while the conversion

under these conditions was 63.8%, with temperature and alcohol excess being the most significant parameters.

Major *et al*.[35] compared ethyl lactate yield in two different types of media: organic solvents (hexane and toluene) and several ionic liquids, with immobilized *Candida antarctica* lipase B (Novozyme 435) and without enzyme. In this sense, at the experimental conditions studied, the ionic liquids Cyphos 163, Cyphos 166, Cyphos 106, Cyphos 102, and Cyphos 110 showed self-catalytic activity without enzyme loading unlike organic solvents. Cyphos 202 was proven to be the best medium for ethyl lactate production owing to highest ethyl lactate yield (95%) after 24 hours of reaction. Moreover, this larger yield was obtained with 20 times less enzyme than toluene, and lactic acid could have been added more concentrated, reducing the amount of ionic liquid. The effect of water content on ethyl lactate yield was also studied. Authors obtained higher ester yield when no water removal was done and concluded that a certain quantity is necessary to avoid the formation of lactic acid polymers (and not only for the proper enzyme functioning) by shifting equilibrium towards dimerization, where water is formed again.

20.4.1.4 Industrial applications of ethyl lactate and potential uses

Ethyl lactate is a flavor compound found naturally in small quantities in a wide variety of foods such as meat, some fruits, soy products and fermented foods such as wine or beer.

This organic ester has recently attracted attention in industry and research due to its physical, chemical and environmental benign properties. On this basis, many processes have been studied and patented in order to reduce its production cost to a competitive level and to promote its use as a replacement of non-green solvents, whose average selling prices have increased drastically due to crude oil price rise.

Ethyl lactate was approved by the U.S. Food and Drug Administration (FDA) as a food additive, and, among other applications, is also used in perfumery, but its main application is as solvent.[6] Ethyl lactate has many powerful features to be used as solvent replacing traditional toxic halogenated and petroleum-based solvents: low toxicity, non-flammability, good solvating ability, is fully biodegradable, non-corrosive, non-carcinogenic, non-ozone depleting and was affirmed GRAS (generally recognized as safe).

In this regard, ethyl lactate is being studied as extraction solvent of food compounds. Several reported potential applications are related with the extraction of carotenoids from different plant matrix,[54,55] with extraction of caffeine from green coffee beans and green tea leaves,[56] the fractionation of edible oil compounds (squalene[57] and tocopherol[58]), the extraction of sclareol from the leaves of *Salvia sclarea* Lamiaceae,[59] the extraction of γ-linolenic acid from *Spirulina microalgae*,[60] the extraction of phytosterols from corn fiber[61] and the extraction of the essential oil from different species of thyme.[62]

Industrially ethyl lactate, along with other lactate ester, is an attractive solvent for the coatings industry (dissolution of coatings for wood, polystyrene, metals and magnetic tapes),[6,63] for the phytosanitary industry (dissolving active pesticide and herbicide compounds) and an excellent cleaner solvent for the polyurethane industry (ethyl lactate can dissolve many polyurethane resins), for electronic and precision parts (where solvent used for these cleaning applications cannot leave residues), for a variety of metal surfaces and can successfully remove oils, paint, dried ink, adhesives and solid fuels.[4,64] This is due to

its high boiling point, low vapor pressure, low surface tension and high solvency power since ethyl lactate can cover a wide range of polarities.[4] These interesting properties cause that ethyl lactate is replacing solvents such as N-methyl pyrrolidone, butan-2-one (MEK), 4-methylpentan-2-one (MIBK), acetone, toluene and xylene.[6]

Ethyl lactate has also been studied as a solvent for soils washing treatments. For instance, the amendment of the highly biodegradable chelating agent ethylenediaminedisuccinic acid (EDDS) with ethyl lactate enhanced the removal efficiency of copper[65] and polycyclic aromatic hydrocarbons (phenanthrene and pyrene)[66] from contaminated soils. In the same way, ethyl lactate has been studied as a candidate to be used for polylactic acid (PLA) dissolution/precipitation due to its partial miscibility with it (PLA is a biodegradable polymer produced from lactic acid which is used in medical and pharmaceutical areas, among others).[67]

Ethyl lactate has both pharmaceutical and cosmetic applications, since ethyl lactate can act as excipient improving the solubility for many classes of biologically active compounds (antihistamines, antivirals, antibiotics…) without undesirable side effects on health.[68] For instance, ethyl lactate can be used in embolic compositions as biocompatible embolic solvent to solubilize biopolymers responsible for embolization of the blood vessel for treating vascular lesions[69] or in veterinary anthelmintic formulations.[70] Ethyl lactate can also act as topical penetration enhancing agent assisting the deep-penetration delivery of cosmetic and pharmaceutical agents into dermal and sub-dermal layers of skin. Moreover this compound has a smooth emollient effect on skin. Thus ethyl lactate can be involved in creams, powders, masks, serums, pastes, sprays and other formulas such as topical inflammation control massage lotions or solubilized vitamin C facial cleanser.[71] Ethyl lactate has also anti-acne properties. Combined with salicylic acid provides a new synergistic treatment for acne[71,72] and it has been observed that ethyl lactate might increase the protection time in insect repellent formulations against *Aedes aegypti* mosquitoes.[73]

Regarding other uses reported, ethyl lactate is being currently explored as green reaction medium for chemical synthesis[74,75] and used as a route to produce 1,2-propanediol, chemical compound mainly used for the production of unsaturated polyester resins, which is actually produced from non-renewable sources on industrial scale.[76,77] Moreover, as already pointed out, ethyl lactate production and subsequent hydrolysis can be used to obtain high-purity lactic acid.[21]

20.4.2 SOLUBILITY AND PHASE EQUILIBRIA DATA

The physicochemical characteristics of ethyl lactate offer diverse solvent properties that cover a large number of solutes as target molecules to extract. Thus, investigation related to the use of ethyl lactate as a green environmentally friendly solvent for the food industry has recently increased. In order to attend the industrial goal, knowledge of the phase behavior of mixtures comprising ethyl lactate is necessary, as is paramount for the design of several technological separation processes.

In this respect, solubility and phase equilibria data of systems comprising ethyl lactate have been recently reported in the literature. For example, Peña-Tejedor *et al.*[13] reported vapor-liquid equilibria (VLE), density and excess volume data of the binary etha-

nol + ethyl lactate at ambient pressure (101.3 kPa). Previous to this work, only a diagram with four experimental VLE data for the system ethanol + ethyl lactate had been reported by Benedict *et al.*[40], who investigated this system at atmospheric pressure just to check for azeotrope formation (fairly common in alcohol + ester mixtures). Both authors[13,40] confirmed the non-existence of azeotrope in the system ethanol + ethyl lactate (Figure 20.4.8). Later, Vu *et al.*[78] presented isothermal VLE data on the mixtures ethanol + ethyl lactate (40.0, 60.1, and 80.2°C) and water + ethyl lactate (40.0 and 60.0°C). While the ethanol + ethyl lactate system showing a slightly non-ideal phase behavior. The water + ethyl lactate system revealed a minimum boiling azeotrope occurring at 5-7 mol% ethyl lactate (Figure 20.4.9). VLE data of the ethyl acetate + ethyl lactate and methyl acetate + ethyl lactate binary mixtures was reported by Resa *et al.*[14] As predictable, both systems presented nearly ideal behavior, with very small (close to zero) excess volumes.

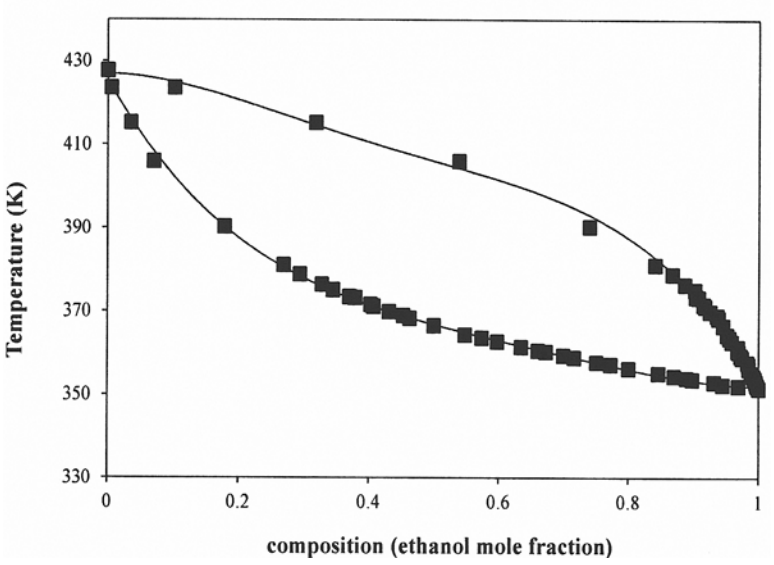


Figure 20.4.8. Vapor-liquid equilibria of the (ethanol + ethyl lactate) system at ambient pressure (101.3 kPa). [Adapted, by permission, from P. Delgado, M.T. Sanz, and S. Beltrán, *Fluid Phase Equilib.*, **255**, 17 (2007).]

VLE measurements of the quaternary system involved in the esterification of lactic acid with ethanol at 101.3 kPa was presented by Delgado *et al.*[79] The esterification reaction is equilibrium-limited and usually does not reach completion. Lactic acid esters are used as powerful high-boiling solvents, and are produced by conventional esterification of lactic acid with the corresponding alcohol.

Liquid-liquid equilibria (LLE) data of systems comprising ethyl lactate was also recently investigated and reported in the literature. For example, Zakrzewska *et al.*[80] presented a comprehensive work in which the LLE of mixtures with ethyl lactate and various hydrocarbons (alkanes, alkenes, cyclohexane, alkyl cyclohexane and terpenes) were explored in the range of temperatures from 273.2 to 320.9K at atmospheric pressure. The respective temperature-composition phase diagrams were constructed and the influence of

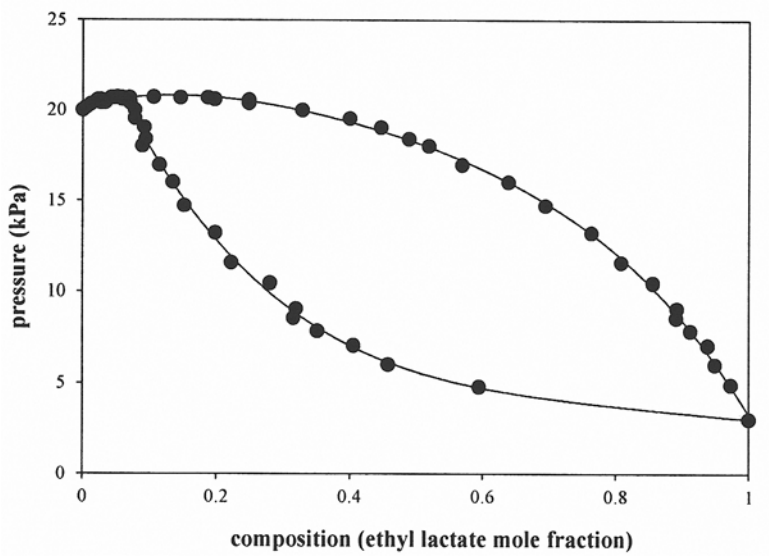


Figure 20.4.9. Vapor-liquid equilibria of the (water + ethyl lactate) system at 333K. [Adapted, by permission, from P. Delgado, M.T. Sanz, and S. Beltrán, *Fluid Phase Equilib*., **255**, 17 (2007).

different variables (temperature, aliphatic chain length, and number of double bonds) on liquid phase behavior was discussed. The binary mixtures of ethyl lactate and low molecular weight alkenes (1-hexene, 1-heptene, 1-octene), cyclohexane and aromatic hydrocarbons (benzene, toluene) have shown complete miscibility in the studied temperature range. On the other hand, mixtures containing ethyl lactate and alkanes (hexane, heptane, octane, isooctane, nonane, decane or dodecane) exhibited partial liquid-liquid miscibility below certain temperature (the upper critical solution temperature, UCST), which increased approximately 7^{o}C per carbon atom in alkane chain. Further, the system ethyl lactate + 1-hexadecene also exhibit UCST behavior. According to the data compiled, the authors established that alkane branching provokes decrease in UCST and higher mutual solubility. Comparing the ethyl lactate + alkane systems with the ethyl lactate + alkene systems, it was concluded that the presence of π-bonds considerably increases mutual solubility.

Other systems investigated included the ethyl lactate and poly(lactic acid) mixture[67] and ethyl lactate + lipid-type substances.[57,58] The lipophilic character of the ester part of ethyl lactate molecule combined with the polarity of its hydroxyl group result in partial liquid-liquid miscibility of this solvent with oils and lipid derivatives, providing novel alternatives for the oil industry processing.

In Sections 20.4.2.1 and 20.4.2.2, the liquid-liquid phase boundary at ambient pressure of binary and ternary systems comprising ethyl lactate and lipid-type substances is reviewed,[57,58] and potential industrial applications based in the LLE observed are introduced. Further, in Section 20.4.2.3, the solubility of several high-value food compounds in ethyl lactate is presented[81] and compared with the solubility in water. Finally, high pres-

sure vapor-liquid phase equilibria of the binary system carbon dioxide + ethyl lactate[82-84] is reviewed in Section 20.4.2.4, and the viability of using ethyl lactate as green co-solvent in supercritical carbon dioxide technology is discussed.

20.4.2.1 Liquid-liquid phase transition of binary and ternary mixtures comprising squalene, olive oil and ethyl lactate

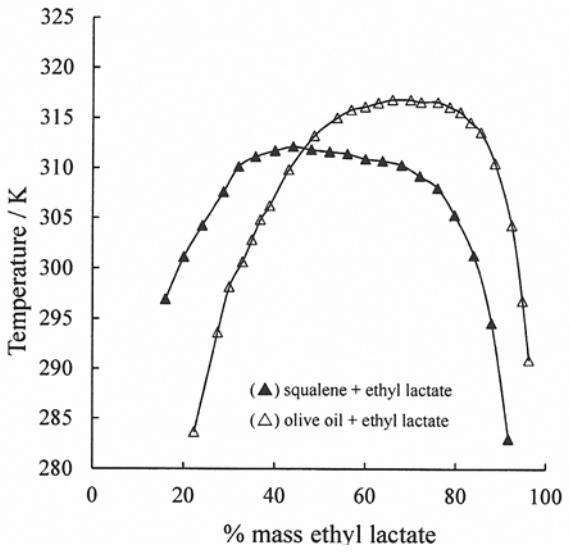


Figure 20.4.10. Transition temperature vs. composition (wt% ethyl lactate) for the binary mixtures (squalene + ethyl lactate) and (olive oil + ethyl lactate) at atmospheric pressure.

Transition temperatures define the liquid-liquid region of a system and are usually determined by the cloud-point method, i.e. observing visually the onset of the phase transition. In general, mixtures of ethyl lactate with lipid-type substances exhibit partial liquid-liquid miscibility below certain temperature (the upper critical solution temperature, UCST) as is depicted in Figure 20.4.10 for the (squalene + ethyl lactate) and (olive oil + ethyl lactate). In these cases, the cloud-point was experimentally measured as follows: a vial containing the sample was introduced in a water bath and was heated to up to temperature at which the system attains homogenous liquid phase; then, heating is turned off and the mixture is slowly cooled while the temperature decrease is continuously registered. The temperature at which turbidity starts to appear in the mixture is the transition temperature, i.e., the temperature at which the homogeneous mixture splits into two liquid phases.

Table 20.4.6 reports the transition temperature of (squalene + ethyl lactate) and (olive oil + ethyl lactate) binary mixtures as a function of the system composition in mass fraction. UCST are 312.2K and 316.9K, for (squalene + ethyl lactate) and (olive oil + ethyl lactate) system, respectively. Figure 20.4.10 shows the temperature-composition liquid-liquid phase diagrams of these mixtures as a function of the ethyl lactate concentration in the corresponding binary mixture.

The two transition curves intersect at 46 wt% of ethyl lactate. Since the main constituents of olive oil are triglycerides (96-98 wt%), mixtures with 0-46% of ethyl lactate will dissolve preferably triglycerides rather than squalene. For example, 30 wt% ethyl lactate completely dissolves triglycerides at 298K while higher temperatures are required to completely dissolve squalene. The opposite holds true for mixtures with concentration of ethyl lactate higher than 46 wt% (right side of Figure 20.4.10). Thus, it can be presumed that, for example, at 313K, 50 to 90 wt% of ethyl lactate added to a triglyceride + squalene

mixture will dissolve squalene better than triglycerides. This suggests that a selective liquid-liquid squalene extraction from squalene + triglyceride mixtures can be successfully realized using ethyl lactate as extraction solvent.

Table 20.4.6. Transition temperatures of (squalene + ethyl lactate) and (olive oil[a] + ethyl lactate) binary mixtures at atmospheric pressure.[57]

Squalene + ethyl lactate		Olive oil + ethyl lactate	
T/K	Squalene, wt%	T/K	Squalene, wt%
283.1	8.28	292.0	3.76
294.7	12.03	297.9	5.18
301.4	16.10	305.4	7.55
305.4	20.27	311.6	11.28
308.1	24.00	314.7	14.47
309.3	28.08	315.7	16.81
310.4	32.08	316.7	19.04
310.8	36.21	317.2	21.43
311.0	40.00	317.7	24.05
311.5	44.04	317.7	27.76
311.7	48.01	317.9	30.07
311.9	51.99	317.9	34.10
312.2	56.00	317.6	37.20
311.8	60.00	317.2	40.15
311.2	64.24	316.9	43.20
310.2	68.12	316.1	46.35
307.7	71.29	314.3	51.34
304.3	76.05	310.9	56.96
301.2	80.10	307.3	61.14
297.0	83.89	305.9	63.17
291.7	88.00	303.9	65.02
		301.7	67.11
		299.2	70.00
		294.7	72.55
		284.7	77.72

[a]Free fatty acid content less than 0.8 wt%.

The transition temperatures of a (squalene + olive oil + ethyl lactate) mixture are given in Table 20.4.7. The mixture contains an olive oil/squalene ratio of 70:30. Figure 20.4.10 depicts the data corresponding to the transition temperature of the (squalene + olive oil + ethyl lactate) mixture vs. ethyl lactate wt%. As can be observed in Figure 20.4.10, the UCST of the ternary system is higher (323.15K) than the corresponding temperatures for squalene + ethyl lactate (312.15K) and olive oil + ethyl lactate (317.15K) mixtures, respectively.

Table 20.4.7. Transition temperatures of the ternary mixture squalene + olive oil (olive oil/squalene ratio = 70:30) and ethyl lactate at atmospheric pressure.[57]

T/K	Squalene, wt%
298.2	95.0
308.4	89.9
313.2	88.0
320.7	79.9
323.2	60.0
320.7	44.9
313.2	38.0
298.2	28.0
293.2	25.1

20.4.2.2 Liquid-liquid equilibria of mixtures containing ethyl lactate, tocopherol and olive oil

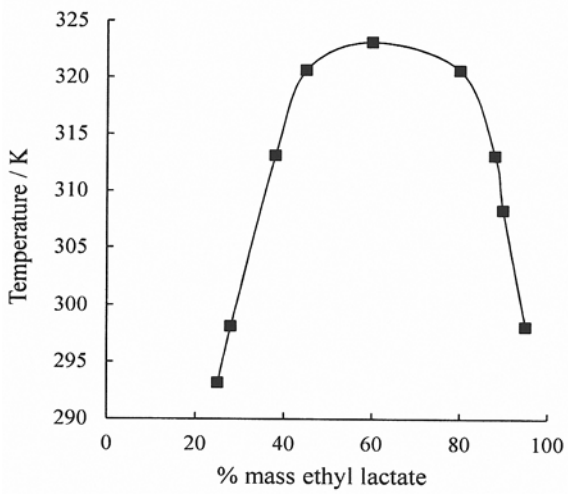


Figure 20.4.11. Transition temperature vs. ethyl lactate content of the ternary mixture squalene + olive oil (olive oil/squalene ratio = 70:30) and ethyl lactate.

Tables 20.4.8 to 20.4.10 and Figure 20.4.11 present the liquid-liquid transition temperatures at ambient pressure of the binary (ethyl lactate + olive oil) system, the pseudo-binary (ethyl lactate + olive oil containing 11.60 wt% of oleic acid) and (ethyl lactate containing 2.80 wt% of water + olive oil). Cloud points at atmospheric pressure were visually detected based on solution turbidity in equilibrium glass cell placed in temperature controlled bath.

The three systems show liquid-liquid partial miscibility by decreasing the temperature at constant pressure, and thus present upper critical solution temperature (UCST) behavior as depicted in Figure 20.4.11. As can be observed from Figure 20.4.12, the addition of oleic acid, which is a naturally occurring olive oil constituent, significantly improves the mutual solubility. The presence of 11.60 wt% of oleic acid in olive oil decreases the UCST 11.2K (see Tables 20.4.8 and 20.4.9).

The observed critical temperature for the binary (ethyl lactate + olive oil) was 311.2K which is lower by approximately 6K comparing with data presented in Section

20.4.2.1. This difference is probably due to the differences in the free fatty acid content of the olive oil employed in these experiments (1.2 wt%) in comparison to the extra-virgin olive oil employed in the experimental data presented in Section 20.4.2.1, which contains less than 0.8 wt% of free fatty acids.

Table 20.4.8. Liquid-liquid transition temperature data of the binary mixture (ethyl lactate + olive oil[a]).[58]

Ethyl lactate, wt%	T/K	Ethyl lactate, wt%	T/K
27.33	281.7	72.44	311.0
38.36	296.6	75.29	311.2
44.79	303.5	77.62	310.9
49.70	306.7	81.75	310.6
56.26	309.2	85.41	309.9
56.78	309.7	88.51	308.6
57.82	309.4	90.81	306.5
61.14	310.1	92.79	303.8
66.69	310.9	94.43	301.6
71.74	310.9	95.11	297.0
		97.23	283.4

[a]Free fatty acid content in olive oil: 1.2 wt%.

Table 20.4.9. Liquid-liquid transition temperature data of the mixture (ethyl lactate + olive oil with 11.6 wt% olive oil).[58]

Ethyl lactate, wt%	T/K	Ethyl lactate, wt%	T/K
34.62	282.7	57.38	299.1
37.15	285.5	59.58	300.0
39.69	288.2	61.29	299.7
41.42	289.8	61.93	300.5
43.92	291.9	66.29	301.2
46.48	293.5	68.34	301.2
48.72	294.7	72.55	301.4
49.18	295.2	76.33	301.2
50.78	295.9	79.20	300.9
52.91	297.1	84.44	298.8
52.93	297.2	88.61	296.7
55.19	298.0	91.53	292.7
56.72	298.5	94.10	286.3

Table 20.4.10. Liquid-liquid transition temperature data of the mixture (ethyl lactate containing 2.80 wt% of water + olive oil).[58]

Ethyl lactate, wt%	T/K	Ethyl lactate, wt%	T/K
19.65	313.2	57.55	349.2
23.17	321.7	61.08	349.7
26.40	328.2	63.01	349.5
29.78	332.7	67.17	348.7
33.05	337.2	70.42	348.1
35.37	339.7	74.03	347.5
39.06	342.8	78.01	346.8
43.06	345.7	82.49	345.2
47.19	347.7	86.38	342.7
52.77	348.8	89.94	338.7
52.86	349.2	93.12	332.7
57.49	349.5	94.90	324.2

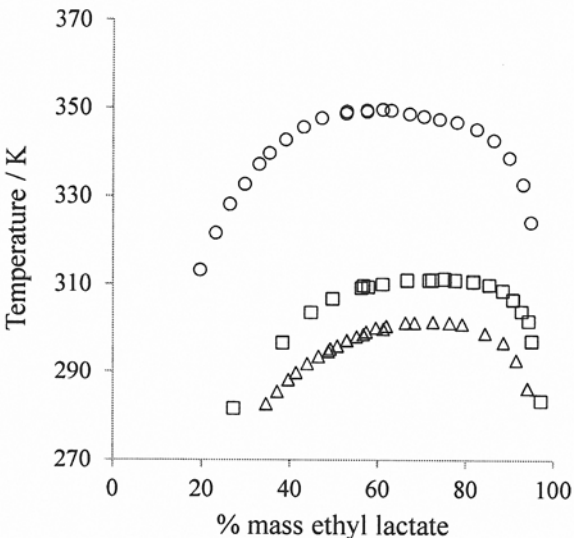


Figure 20.4.12. Temperature vs. ethyl lactate mass fraction phase diagrams at atmospheric pressure for the mixtures (□) (ethyl lactate + olive oil), (Δ) (ethyl lactate + olive oil with 11.60 wt% of oleic acid) and (O) (ethyl lactate containing 2.80 wt% of water + olive oil).

On the other hand, water has a strong anti-solvent effect in this type of mixture. For example, the presence of 2.80 wt% of water in ethyl lactate increased the UCST for approximately 39K, producing higher immiscibility of the system. Figure 20.4.13 presents the UCST of the (olive oil + ethyl lactate) mixture as a function of the water content in ethyl lactate The linear regression of data presented in Figure 20.4.13 reveal that the presence of 0.1 wt% water in ethyl lactate solvent leads to 1.4K increase of UCST.

Mixtures of (α-tocopherol + olive oil) and (α-tocopherol + ethyl lactate) show complete miscibility in the temperature range from 270 to 360K as reported by Vicente *et al.*[58] while the ternary (α-tocopherol + olive oil + ethyl lactate) system present liquid-liquid partial miscibility in this temperature range.

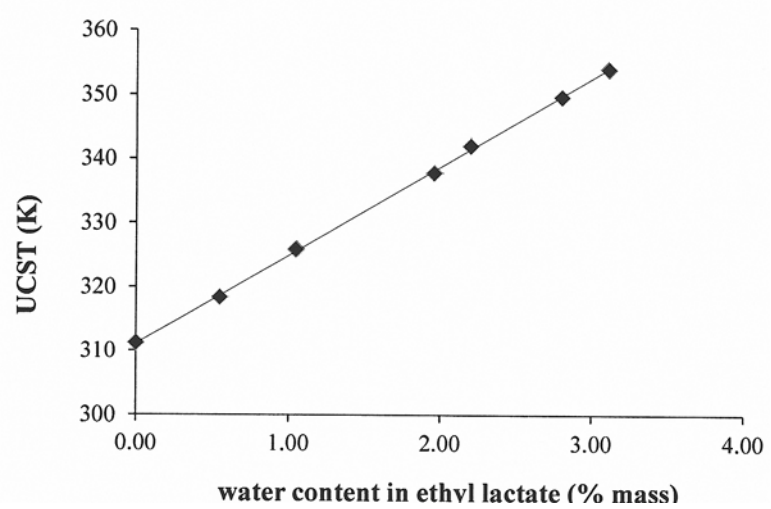


Figure 20.4.13. Effect of the presence of water in the ethyl lactate solvent on the UCST of the mixture (ethyl lactate + olive oil): (◆) experimental data; (—) trend line.

The cloud point data, which define the liquid-liquid phase boundary of the ternary (α-tocopherol + olive oil + ethyl lactate) mixture, is given in Table 20.4.11 at two different temperatures, 298.2K and 288.2K. Additionally, Table 20.4.12 presents phase liquid-liquid composition data for both ethyl lactate-rich and olive oil-rich phases at equilibrium, as well as respective overall compositions.

From Tables 20.4.11 and 20.4.12 it can be concluded that the mutual solubility of the ternary system decreases with temperature. This suggests that low temperatures are more suitable for extraction of α-tocopherol from olive oil using ethyl lactate as a solvent.

Table 20.4.11. Liquid-liquid transition temperature data for (α-tocopherol + olive oil + ethyl lactate) at 298.2K and at 288.2K.[58]

298.2K			288.2K		
Composition, wt%			Composition, wt%		
α-tocopherol	olive oil	ethyl lactate	α-tocopherol	olive oil	ethyl lactate
8.2	44.5	47.3	7.3	57.6	35.2
11.1	35.7	53.2	11.8	46.6	41.6
8.0	13.9	78.1	13.7	27.9	58.4
5.0	10.1	84.9	7.2	6.9	85.9
4.3	54.1	41.7	13.4	40.9	45.8
10.9	26.6	62.5	13.7	21.6	64.7
10.2	20.1	69.7	11.7	13.5	74.8
			11.2	47.4	41.5
			13.3	37.2	49.6

Table 20.4.12. Tie-line data for (α-tocopherol + olive oil + ethyl lactate) system at 298.2K and 288.2K (atmospheric pressure). w_i: mass fraction; 1: α-tocopherol; 2: olive oil; 3: ethyl lactate.[58]

Olive oil-rich phase			Ethyl lactate-rich phase		
w_1	w_2	w_3	w_1	w_2	w_3
T = 298.2K					
0.0335	0.5549	0.4116	0.0295	0.0799	0.8906
0.0493	0.5388	0.4119	0.0519	0.1028	0.8453
0.077	0.4898	0.4332	0.0686	0.1428	0.7886
T=288.2K					
0.0521	0.6075	0.3404	0.0675	0.074	0.8585
0.1011	0.4973	0.4016	0.1286	0.1427	0.7287

Taking into account the data provided in Table 20.4.12, the partition coefficient:

$$K_i = \frac{w_{i,\text{ ethyl lactate-rich phase}}}{w_{i,\text{ oil-rich phase}}} \quad [20.4.2]$$

of α-tocopherol and olive oil can be calculated. The partition coefficient of α-tocopherol is very close to 1 at 298.2K, while slightly higher values of 1.3 were obtained at 288.2K. In the case of partition coefficient of olive oil, no significant difference was observed when lowering the temperature. Thus, the separation factor between these two substances:

$$\alpha = \frac{K_{\alpha\text{-tocopherol}}}{K_{\text{olive oil}}} \quad [20.4.3]$$

is higher at lower temperature. That is, low temperatures are more suitable for extraction of α-tocopherol from olive oil using ethyl lactate as a solvent.

20.4.2.3 Solubilities of solid high valued compounds in ethyl lactate

High-value compounds derived from natural sources are becoming targets of industrial importance due to the increased perception of their health benefits associated with their biological activities. For example, several hydroxycinnamic acids, such as ferulic and caffeic acids, have shown good potential in the prevention of chronic illnesses such as cardiovascular diseases and cancer.[85,86] Another phenolic compound, with high biological activity, is vanillic (an hydroxybenzoic acid) which is known for its antisickling and anthelmintic activities.[87,88] Thymol, a compound characteristic of essential oils, has been identified as an effective anti-bacterial, and demonstrated dose-dependent cytotoxic effects on acute promyelotic leukemia cells.[89]

Caffeine is one of the most widely consumed and studied alkaloids. Although research results are controversial, it is believed that low to moderate caffeine intake is generally associated with improvements in alertness, learning capacity, exercise performance,

and possibly even in mood.[90] Furthermore, it is also used as an additive in pain medications.

Tables 20.4.13 to 20.4.17 present the solubility of several high-value compounds, namely caffeine, vanillic acid, ferulic acid, caffeic acid and thymol, in liquid ethyl lactate in the temperature range of 293.2-343.2K. The chemical structures of these compounds are depicted in Figure 20.4.14. Solubilities in both water-saturated (1.4 wt%) and dried (0.03 wt%) ethyl lactate are given in the tables, since the hydroscopic character of ethyl lactate makes important to understand the effect of small amounts of water on solute solubility.

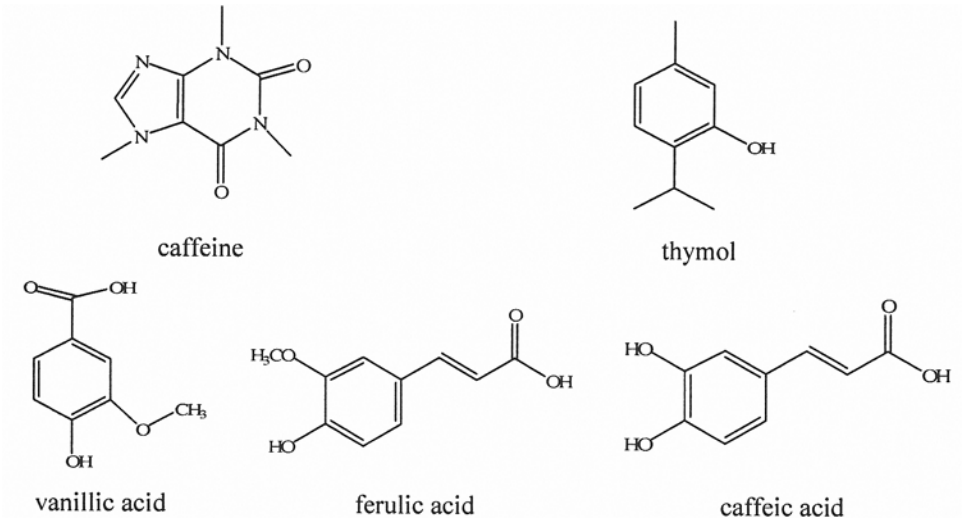


Figure 20.4.14. Chemical structures of solutes referred in Tables 20.4.13 to 20.4.17.

Table 20.4.13. Experimental solubilities of caffeine in ethyl lactate.[81]

1.40 wt% water in ethyl lactate		< 0.03 wt% water in ethyl lactate	
T/K	**Caffeine, wt%**	**T/K**	**Caffeine, wt%**
298.2	3.12	296.2	2.35
313.2	4.92	303.1	3.21
328.2	6.69	312.7	4.09
343.2	8.09	323.0	5.14
		333.3	6.63

Table 20.4.14. Experimental solubilities of vanillic acid in ethyl lactate.[81]

1.40 wt% water in ethyl lactate		< 0.03 wt% water in ethyl lactate	
T/K	Vanillic acid, wt%	T/K	Vanillic acid, wt%
298.2	3.80	296.2	3.93
313.2	4.98	303.1	4.51
328.2	6.73	312.7	5.31
343.2	8.11	323.0	6.20
		333.3	7.58

Table 20.4.15. Experimental solubilities of ferulic acid in ethyl lactate.[81]

1.40 wt% water in ethyl lactate		< 0.03 wt% water in ethyl lactate	
T/K	Ferulic acid, wt%	T/K	Ferulic acid, wt%
298.2	12.55	296.2	4.47
313.2	14.56	303.1	5.61
328.2	16.33	312.7	6.85
343.2	17.99	323.0	8.36
		333.3	9.71

Table 20.4.16. Experimental solubilities of caffeic acid in ethyl lactate.[81]

1.40 wt% water in ethyl lactate		< 0.03 wt% water in ethyl lactate	
T/K	Caffeic acid, wt%	T/K	Caffeic acid, wt%
298.2	1.95	296.2	1.35
313.2	2.50	303.1	1.56
328.2	3.06	312.7	1.80
343.2	3.47	323.0	2.15
		333.3	2.59

The relative affinity of the studied solutes to ethyl lactate follows the order: thymol >> ferulic acid > vanillic acid > caffeine > caffeic acid. As expected, solubilities of all studied solutes in ethyl lactate were moderately enhanced by temperature rise. Thymol is extremely soluble in ethyl lactate, reaching ca. 91 wt% at 317.8K which can be explained by its relatively low melting point and enthalpy of fusion.

The chemical structures of ferulic acid and caffeic acids are quite similar (Figure 20.4.14) while their solubility in ethyl lactate is quite different (Tables 20.4.15 and 20.4.16). For example, at 333.3K the solubility of ferulic acid is 9.71 wt%, and that of caf-

feic acid is 2.59 wt%. That is, the substitution of one hydroxyl group of caffeic acid by a methyl ether group enhances the solubility significantly. Furthermore, the solubilities observed for vanillic and ferulic acids at 333.3K were 7.58 and 9.71 wt%, respectively, indicating that the presence of a longer acid alkyl chain in the phenolic acid only slightly increases solubility.

Table 20.4.17. Experimental solubilities of thymol in ethyl lactate.[81]

1.40 wt% water in ethyl lactate		**< 0.03 wt% water in ethyl lactate**	
T/K	**Thymol, wt%**	**T/K**	**Thymol, wt%**
301.4	72.36	301.0	72.39
304.3	75.25	303.5	74.56
307.5	78.74	307.5	78.60
307.8	78.90	308.4	79.96
308.3	79.33	309.3	81.29
316.5	90.12	311.0	82.74
318.6	92.32	313.3	85.83
		317.8	90.95

The solubility data reviewed in Tables 20.4.13 to 20.4.17 reveal that the solubility of solutes was differently influenced by the presence of water in ethyl lactate solvent. For example, the solubility of thymol was not changed by water, that of vanillic acid and caffeine was only slightly influenced, and a significant increase of the solubility of ferulic and caffeic acids were observed when the content of water in ethyl lactate was increased. Taking into account a low solubility of ferulic and caffeic acids in water, this solubility enhancement suggests a co-solvent effect which may have implications in potential extraction processes.

Table 20.4.18 show a comparison between the solubility at ambient temperature (298K) and pressure of several high valued food grade substances in ethyl lactate and in the most popular food grade solvent, i.e., water. As can be observed, caffeine has similar solubility in both solvents (around 2 wt%), while the solubilities of phenolic acids and catechins are considerably higher in ethyl lactate than in water. As it will be discussed later, these physical characteristics can be exploited to study the development of novel extraction processes for the food industry.

Table 20.4.18. Solubility (wt%) of several food substances in ethyl lactate and water at ambient temperature and pressure (298K and 101.3 kPa).

	Ethyl lactate	Water
Caffeic acid	1.4	0.1
caffeine	2.3	2.2
Vanillic acid	3.9	0.2
Ferulic acid	4.5	0.6
(+)-catechin	2.3	0.2
Chlorogenic acid	9.7	2.5

20.4.2.3.1 Thermodynamic modeling

The development of appropriate thermodynamic models to represent or even predict solubility and phase equilibria of mixtures is still a challenging task. Knowledge of solubilities and equilibrium compositions are essential for evaluating the feasibility of separation process. The progress of these models allows the development of computer aided tools for the design, simulation, and optimization of viable separation processes.

In the case of modeling the solubility of a pure solid solute in a liquid solvent (ethyl lactate), the equi-fugacity equilibrium condition for solvent (1) and the solid (2) is:

$$f_2^S = f_2^L \qquad [20.4.4]$$

where: f_2^S is the fugacity of the solute in the solid phase and f_2^L is the fugacity of the solute in the liquid ethyl lactate phase. The solute fugacity in the liquid phase can be referred to as the fugacity of the pure solute in liquid state f_2^0:

$$f_2^L = \gamma_2 x_2 f_2^0 \qquad [20.4.5]$$

where: γ_2 is the activity coefficient of the solute in the liquid phase, x_2 is its molar fraction (solubility) and f_2^0 is the liquid-phase standard state fugacity that typically is taken as the pure-liquid fugacity at the system temperature and at pure-liquid vapor pressure, with the corresponding corrections for pure-fluid vapor-phase non-ideality and for the effect of total pressure.

Replacing Eq. [20.4.5] in Eq. [20.4.4] the following relation for the solubility is obtained:

$$\ln x_2 = \ln((f_2^S / f_2^0) - \ln\gamma_2) \qquad [20.4.6]$$

When the mixture temperature is lower than the solute triple point temperature, f_2^0 stands for the pure solute in a hypothetical liquid state. Additionally, for most substances, there is a little difference between the triple point temperature and the normal melting temperature. Thus, the ratio f_2^S / f_2^0 can be calculated as follows:

$$\ln(f_2^S/f_2^0) = -\frac{\Delta H_{m_2}}{RT_{m_2}}\left(\frac{T_{m_2}}{T}-1\right)+\frac{\Delta C_{p_2}}{R}\left[\left(\frac{T_{m_2}}{T}-1\right)+\ln\left(\frac{T}{T_{m_2}}\right)\right] \qquad [20.4.7]$$

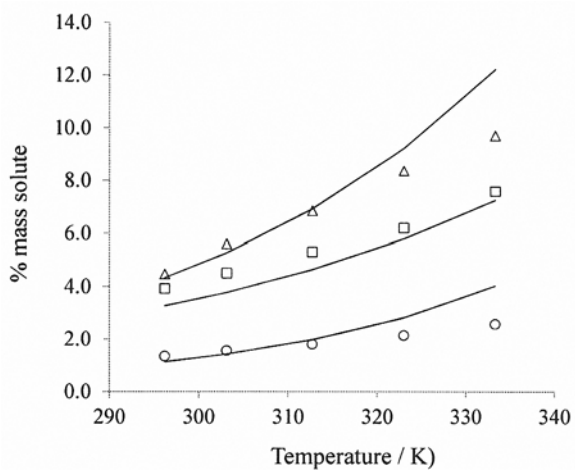


Figure 20.4.15. Solubilities of phenolic acids in ethyl lactate: (□) vanillic acid, (Δ) ferulic acid and (O) caffeic acid. Solid lines: thermodynamic modeling using UNIFAC.

where T_{m_2} and ΔH_{m_2} are, respectively, the solute normal melting temperature and enthalpy of fusion, and ΔC_{p_2} is the difference between the heat capacity of the pure liquid and solid solute.

Eq. [20.4.6] and Eq. [20.4.7] were applied to calculate the solubility of phenolic acids (caffeic, vanillic and ferulic acids) in pure ethyl lactate. The solute activity coefficients were calculated using UNIFAC group contribution method.[91] The enthalpies of fusion, melting temperatures and differences in heat capacities of the solutes, along with the UNIFAC group interactions parameters were taken from the literature.[81] Appendix B includes pure solute physical properties together with UNIFAC model equations and group interactions parameters, which are required to carry out the solubility calculations.

Figure 20.4.15 shows a comparison between the experimental solubility given in Tables 20.4.14 to 20.4.16 (ethyl lactate with less than 0.03 wt% of water) and the solubility calculations performed with the UNIFAC model. The absolute average deviations (AAD) were calculated as follows:

$$AAD(\%) = \frac{1}{NP}\sum_i \frac{\left|w_i^{calc} - w_i^{exp}\right|}{w_i^{exp}} \times 100 \qquad [20.4.8]$$

where w_i^{calc} and w_i^{exp} are the calculated and experimental mole fraction solubilities, respectively, and NP is the number of available solubility points. The AAD obtained were 11.4% for vanillic acid, 9.6% for ferulic acid and 24.7% for caffeic acid.

According to the deviations obtained, it can be concluded that a satisfactory prediction of the solubility of solid phenolic acids in ethyl lactate can be achieved using the equifugacity equilibrium condition and UNIFAC group contribution approach to calculate the activity coefficient of the phenolic acid in the liquid ethyl lactate-rich phase.

20.4.2.4 Phase behavior of ethyl lactate + CO_2 mixture

Ethyl-lactate is a novel ecofriendly solvent with potential applications in supercritical fluid technology, as a co-solvent of carbon dioxide, in high pressure chemical reactions, supercritical extraction processes and/or anti-solvent precipitation processes. In view of this, knowledge of the phase behavior of (ethyl lactate + CO_2) binary is essential for the modeling and design of such processes.

The first equilibrium data on the ethyl lactate + CO_2 system were reported by Chylinski and Gregorowicz[82] in 1998, and correspond to the solubility of ethyl lactate in the CO_2-rich supercritical phase at three different temperatures (311, 318 and 323K) and pressures up to 8.1 MPa. Later, Villanueva *et al.*[17] measured the solubility of CO_2 in the ethyl lactate-rich phase at the same temperatures and pressure range (see Table 20.4.19).

Additionally, Cho *et al.*[83] also provided phase equilibrium data of the ethyl lactate + CO_2 mixture and more recently, Paninho *et al.*[84] presented the equilibrium compositions of both liquid and supercritical phases (see Figure 20.4.16) in a wide range of temperatures (313-393K) and pressures (0.4-17 MPa).

Table 20.4.19. Liquid phase composition of the binary system (ethyl lactate + CO_2) at T = (311, 318 and 323)K.[17]

T K	p MPa	CO_2 wt%	T K	p MPa	CO_2 wt%	T K	p MPa	CO_2 wt%
311	1.2	3.2	318	1.2	2.9	323	1.2	2.9
311	2.0	7.4	318	2.8	9.6	323	2.0	6.5
311	2.8	10.3	318	3.8	13.0	323	2.8	8.7
311	3.8	14.7	318	6.2	21.0	323	3.9	11.9
311	4.5	18.8	318	7.0	26.5	323	4.9	14.0
311	6.2	28.8	318	7.8	31.8	323	6.2	17.9
311	6.8	34.4	318	8.1	35.8	323	7.0	19.8
311	7.0	35.4				323	8.1	29.9
311	7.8	41.9						

The data reported[82-85] show that ethyl lactate weight fractions in the liquid phase increase when temperature increases and pressure decreases. On the other hand, the weight fractions of ethyl lactate in the CO_2-rich phase increase with pressure, and the effect of temperature depends on pressure. Nevertheless, some discrepancies could be observed between the data reported by Villanueva *et al.*[17] and the liquid phase compositions reported by the other authors[83,84] which could be due to the difference in the experimental methodology used or even in the quality (purity) of the ethyl lactate solvent employed. As mentioned before, ethyl lactate is a very hydroscopic substance and the presence of water can greatly influence solubility and phase equilibria measurements (see for example Figure 20.4.12).

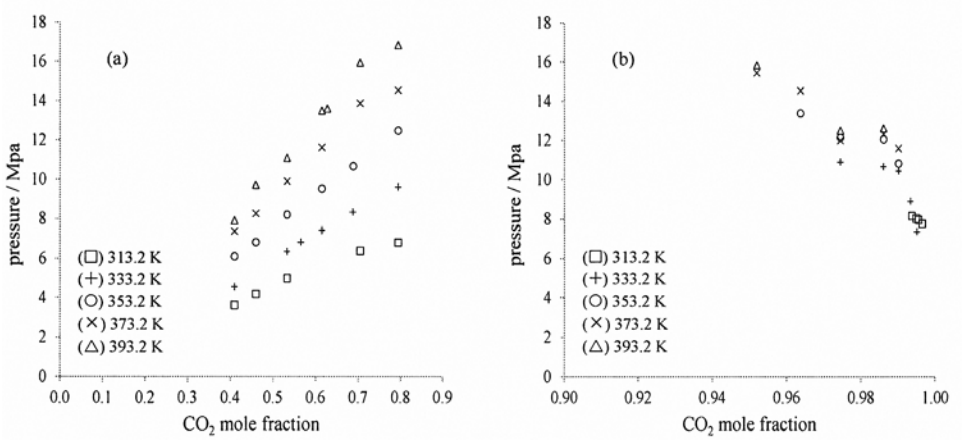


Figure 20.4.16. Vapor-liquid equilibria representation of the (ethyl lactate + CO_2) mixture. (a) Liquid phase; (b) vapor phase. [Adapted, by permission, from A.B. Paninho, A.V.M. Nunes, A. Paiva, and V. Najdanovic-Visak, *Fluid Phase Equilib*., **360**, 129 (2013).]

Paninho *et al.*[84] compared the solubility of CO_2 in ethyl lactate with the solubility of CO_2 in common solvents, such as ethanol and ethyl acetate. The relative affinity observed follows the order: ethyl acetate > ethyl lactate > ethanol. Besides the utilization of ethyl lactate as cosolvent of CO_2 supercritical processing, as will be discussed in Section 20.4.3, the high solubility of CO_2 in ethyl lactate promotes different approaches in the perspective of expanded liquid solvents, i.e. an organic liquid in which CO_2 has been dissolved. The exploitation of these new class of solvents is based in the possibility of modify different liquid physical properties, such as viscosity, surface tension, diffusion rates, solubility of reagents, catalysts and substrates, density and polarities, by dissolving CO_2 at high pressure. In this context, ethyl lactate is a good candidate, with the additional advantage of being itself a green solvent.

20.4.3 NOVEL FOOD APPLICATIONS OF ETHYL LACTATE

20.4.3.1 Fractionation of edible oil compounds

In Section 20.4.2 it was established that the mixtures of ethyl lactate with lipid type substances usually present partial liquid-liquid miscibility with upper critical solution temperature (UCST) critical point. This property could be exploited to study and develop new separation processes for the edible oil industry. In this section two particular applications will be reviewed: the recovery of squalene from olive oil deodorizer distillates and the extraction of tocopherol from olive oil. Both case studies are based in the liquid-liquid equilibria (LLE) data presented in Section 20.4.2.

20.4.3.1.1 Recovery of squalene from olive oil deodorizer distillates

Squalene (2,6,10,15,19,23-hexamethyl tetracosahexane) is a very valued substance in both the cosmetic and pharmaceutical industries. The primary natural source of squalene is shark liver oil, but it can also be found as a minor constituent in olive oil. Thus, residues of

the olive oil deodorization process are viable raw materials for the production of squalene from vegetal sources. Olive oil deodorizer distillates (OODD) may contain up to 30 wt% of squalene, together with free fatty acids (30-40%), fatty acid esters, (20-30%) and minor amounts of other compounds such as mono- and diglycerides, sterols, tocopherols, etc. Esterification of OODD would drive to a mixture mainly comprised by triglycerides and squalene. Then the recovery of squalene from the triglyceride-rich mixture could be accomplished by different methods, including supercritical fluid extraction.

The possibility of a liquid-liquid extraction of squalene from squalene + triglyceride mixtures using ethyl lactate was first reported by Hernández *et al*.[57] Taking into account the liquid-liquid phase transition measured (Figure 20.4.10), these authors determined the liquid-liquid equilibrium compositions of two selected systems, namely, a mixture with 90 wt% ethyl lactate and another one with 80 wt% ethyl lactate, at two selected temperatures, 298.15K and 313.15K. Both temperature-composition conditions were selected to promote the dissolution of squalene in the ethyl lactate rich phase (see Figure 20.4.10).

The compositions of the equilibrium liquid phases obtained are given in Table 20.4.20 (L_1 and L_2) and reveal that in both cases a selective extraction of squalene could be attained. Particularly, at 298.15K (ambient temperature) L_2 contains 42.0 wt% of squalene (solvent free basis). This means that a 1.4 fold increase of squalene was obtained.

Table 20.4.20. Compositions of equilibrium liquid phases (L_1 and L_2) of the system squalene+triglyceride (S+T) (30 wt% squalene) and ethyl lactate (EL).[57]

T/K	EL/S+T	L_1 (triglyceride-rich phase)			L_2 (ethyl lactate-rich phase)		
		wt%			wt%		
		S	T	EL	S	T	EL
298.2	9	12.5	59.5	28.0	2.1	2.9	0.95
313.2	4	12.8	49.2	38.0	4.1	7.9	0.88

Table 20.4.21 shows the partition coefficients k_i^{eq} (wt% in L_2/wt% in L_1) for the squalene (SQ) and triglycerides (TG) compounds, calculated on ethyl lactate free basis, together with the corresponding separation factors ($R = k_{SQ}^{eq}/k_{TG}^{eq}$). As can be observed from the values obtained, a high separation factor ($R > 3$) was obtained at 298.15K. Then, a selective recovery of squalene from squalene + triglyceride mixtures using ethyl lactate is feasible at 298.15K (no heating requirements) and providing an ethyl lactate/squalene-triglyceride ratio lower than 10 (low amount of solvent requirement).

Table 20.4.21. Partition coefficients k_{SQ}^{eq} and k_{TG}^{eg} (ethyl lactate-free basis) and separation factor $\alpha = k_{SQ}^{eq}/k_{TG}^{eq}$ of the squalene (SQ) + triglyceride (TG) (30 wt% squalene) and ethyl lactate (EL) mixture.

T/K	k_{SQ}^{eq}	k_{TG}^{eq}	$\alpha = k_{SQ}^{eq}/k_{TG}^{eq}$
298.15	2.419	0.702	3.447
313.15	1.615	0.840	1.922

20.4.3.1.2 Extraction of tocopherol from olive oil

Tocopherols are substances with high biological activities, very well-known as vitamin E. Currently most of tocopherols are obtained by vacuum distillation of the residues produced in vegetable oil refining. This procedure requires several steps and involves abundant amounts of organic solvents. Thus, in the search for less costly and ambient friendly alternatives, ethyl-lactate appears as a very suitable solvent.

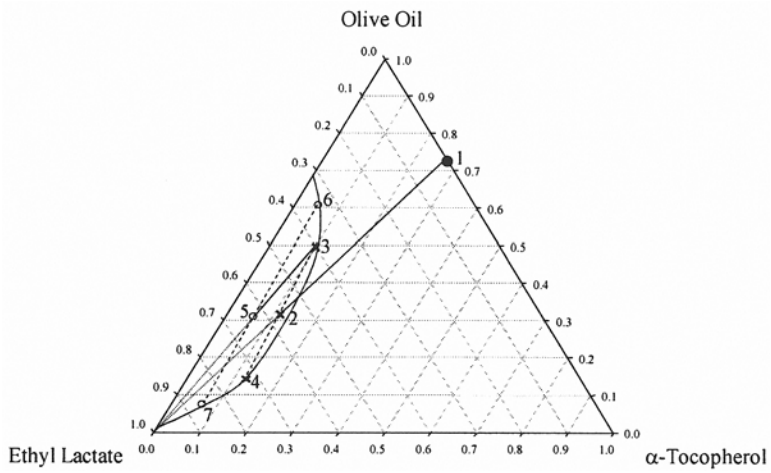


Figure 20.4.17. Liquid-liquid equilibria of two-step extraction process to recover tocopherols from olive oil using ethyl lactate. [Adapted, by permission, from G. Vicente, A. Paiva, T. Fornari, and V. Najdanovic-Visak, *Chem. Eng*. J., **172**, 879 (2011).]

Tables 20.4.11 and 20.4.12 present liquid-liquid equilibrium data for the ternary (ethyl lactate + olive oil + α-tocopherol) system at 288.2 and 298.2K. Considering these data, Vicente *et al*.[58] suggested a liquid-liquid countercurrent process to extract α-tocopherol from olive oil using the green solvent ethyl lactate, the tie-line data of Table 20.4.12 allows the calculation of extraction yield as presented in Figure 20.4.17 for the temperature of 288.2K. A two-step extraction process is considered. If the (α-tocopherol + olive oil) raw material contains 27 wt% of α-tocopherol (point 1 in Figure 20.4.17) and is contacted with sufficient ethyl lactate, a biphasic mixture is formed (point 2) comprising an oil-rich phase (point 3) and ethyl lactate-rich phase (point 4, which is connected by the

respective tie-line with point 3). If we further contact the oil rich phase with additional ethyl lactate, the resulting mixture (point 5) splits into two phases: oil-rich phase (point 6) and ethyl lactate-rich phase (point 7).

The tie-line data in Table 20.4.12 allow calculating the extraction yield of the proposed two-step countercurrent extraction process according to:

$$Y = \frac{\text{mass of extracted } \alpha - \text{tocopherol}}{\text{mass of } \alpha - \text{tocopherolin raw material}} \cdot 100\ (\%) \qquad [20.4.9]$$

The extraction yield of the first-step is ca. 60% and that of second-step is 65%, while extraction yield of the two-step process is 85%. Thus, both yield and separation factors obtained indicate good selectivity of ethyl lactate used as a solvent to extract α-tocopherol from α-tocopherol + triglyceride mixtures. Hence, the results presented demonstrate the viability of liquid-liquid countercurrent process to extract α-tocopherol from olive oil using green ethyl lactate extraction solvent.

20.4.3.2 Decaffeination of coffee beans and green tea

Among natural sources of caffeine, two very popular drinks namely coffee and green tea, should be quoted. Coffee plants with large economic and commercial importance are *Coffea arabica* and *Coffea robusta*. Furthermore, Arabica coffee beans are preferred by consumers and are considered of superior quality at the international market. Caffeine content in Arabica green beans is around 1 wt%.[92] Another plant that contains caffeine is green tea (*Camellia sinensis*), which has been a much consumed drink in Asian countries over years but nowadays is very popular all over the world. Green tea leaves contains around 20-40 mg/g of caffeine and 190-260 mg/g of catechins.[93,94] Catechins are recognized to be the most beneficial bioactive compounds of green tea, including antioxidant, anticancer, anti-inflammatory, antibiotic and antiviral effects.[95,96]

Indeed, the most novel approach to decaffeinate green coffee beans, which has attained industrial application, is supercritical fluid extraction (SFE) using carbon dioxide (CO_2).[97] Supercritical CO_2 is selective for the extraction of caffeine, there is no associated waste treatment of a toxic solvent and extraction times are moderate. Moreover, supercritical CO_2 coupled with ethanol or water was investigated to extract caffeine from green tea leaves.[93,98]

Traditional coffee decaffeination was attained using liquid solvents such as benzene, chloroform, trichloroethylene and dichloromethane. However, evidence suggested that chlorinated solvents are carcinogenic and thus, their use was firmly reduced. Extraction of caffeine with water (a green solvent) requires a two-step process because many flavor and aroma substances are, as caffeine, very soluble in water. Ethyl acetate (obtained from synthesis) is also employed in coffee decaffeination and is much more selective for caffeine and thus, extraction can be accomplished in a single-step contact process.

As in the case of green coffee beans, the presently used commercial methods for decaffeination of green tea leaves have been liquid solvent extraction, using chlorinated solvents, ethyl acetate, acetone, ethanol and acetonitrile. Although good decaffeination can be achieved using these solvents, catechins are also considerably co-extracted and thus, the value of green tea as a functional healthy drink is reduced.

The solubility of caffeine in ethyl lactate is very similar to the values reported for the solubility of caffeine in water (see Tables 20.4.13 and 20.4.18). Thus, the agrochemical ethyl lactate can be employed as an environmentally friendly solvent to extract caffeine from natural matter, as proposed Villanueva *et al.*[56] In their work, Pressurized Liquid Extraction (PLE) of green coffee beans and green tea leaves using ethyl lactate was investigated and compared with the use of other liquid solvents, such as ethyl acetate and ethanol. Static extraction assays (one step during 10 min) were carried out in an Accelerated Solvent Extraction (ASE) system at high extraction temperatures (100, 150 and 200ºC) providing a pressure high enough so as to maintain the liquid state of the solvent. The high temperatures applied in PLE favor solutes solubility. Furthermore, a compression effect is made on the vegetal particle, which also contributes to improved extraction. Using this alternative liquid solvent extraction, lower amount of solvent is required, extraction is faster, higher yields are attained and the loss of volatiles is minimized.[99]

High recovery of caffeine was found in the extracts produced using ethyl lactate (see Table 20.4.22) what demonstrates the potential use of this green solvent for the extraction of caffeine from different vegetable sources.

Additionally, Villanueva *et al.*[56] studied the co-extraction of other valuable compounds of coffee, such as phenolic compounds (mainly chlorogenic acids) and oils. These substances have an important role during coffee roasting as precursors of key compounds of coffee flavor and aroma and thus, their removal from natural matter during extraction should be minimized.

In this respect, Villanueva *et al.*[56] concluded that with increased extraction temperature the concentration of phenolic compounds was increased in the samples while decreased concentrations of lipid-type substances was also determined. For all solvents studied, including ethyl lactate, the co-extraction of phenolic compounds and coffee oil represent, respectively, less than 28% and 21% of the corresponding amounts present in the raw material. Moreover, in comparison with ethyl acetate, ethyl lactate extracted similar amounts of coffee oil but almost twice the amount of phenolic compounds, what can be attributed to the higher polarity of ethyl lactate due to the hydroxyl group present in its chemical structure.

Table 20.4.22. Caffeine recovery (mg caffeine/g natural matter) obtained in the PLE of green coffee beans and green tea leaves.[56]

Natural matter	Extraction temperature		
	100ºC	**150ºC**	**200ºC**
Solvent: ethyl lactate			
entire green coffee beans	0.08	0.78	5.36
green tea leaves	11.98	18.92	22.58
Solvent: ethanol			
entire green coffee beans	0.21	1.06	4.40
green tea leaves	19.62	23.45	23.91

Table 20.4.22. Caffeine recovery (mg caffeine/g natural matter) obtained in the PLE of green coffee beans and green tea leaves.[56]

Natural matter	Extraction temperature		
	100°C	**150°C**	**200°C**
Solvent: ethyl acetate			
entire green coffee beans	0.16	0.59	3.83

A comparison between the caffeine and catechins extracted from green tea leaves by PLE is given in Figure 20.4.18 as a function of extraction temperature. As can be observed in the figure, at low temperatures the recovery of alkaloids using water is higher than that achieved using ethyl lactate, but at high temperatures the alkaloid recoveries obtained with both solvents become similar (> 50%). With respect to catechins, high temperatures (> 150ºC) produce degradation of these compounds. Furthermore, in general, catechin recoveries are considerably lower than those of alkaloids (< 18%) but are lower using ethyl lactate, what can be considered a good starting point to design a high quality decaffeinated green using ethyl lactate solvent.

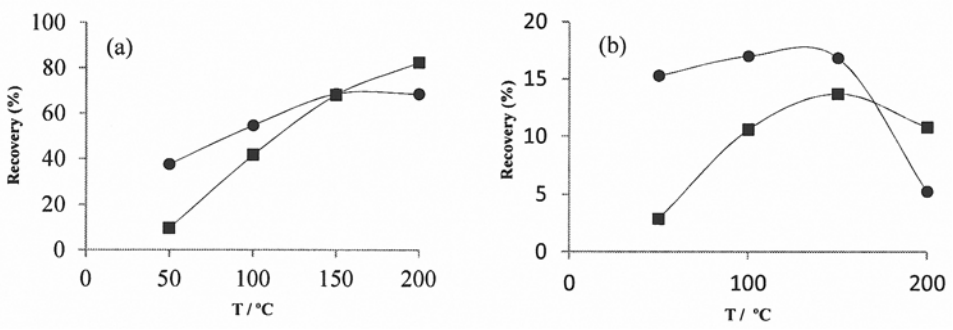


Figure 20.4.18. Recovery (mass extracted / mass in natural matter x 100) of (a) alkaloids and (b) catechins in the PLE of green tea leaves using (●) water or (■) ethyl lactate.

20.4.3.3 Extraction of carotenoids from different plant matrix

The use of ethyl lactate as extraction solvent of carotenoids has been reported by several authors. The obtained results invite to consider ethyl lactate as a potential substitute for toxic petroleum-derived solvents in the recovery of carotenoids from plant matrix.

20.4.3.3.1 Extraction of β-carotene, lutein and lycopene from carrots, white corn and tomato

Carotenoids are tetraterpenoid organic pigments that are found in plants, algae, some bacteria, and fungi. Carotenoids can be divided into two classes of compounds: carotenes, which are hydrocarbon compounds without oxygen in their chemical structure and xanthophylls, which are the oxygenated compounds. Lycopene, α- and β-carotene, lutein, and zeaxanthin are carotenoids found in relatively larger amounts in fruits and vegetables.[100]

Ishida and Chapman[54] studied ethyl lactate as a green solvent to extract carotenoids from several vegetable sources. The authors determined the carotenoid yield (μg of carotenoid per g of dry weight) at different conditions and the results were compared with extraction carried out with ethanol (another green solvent), ethyl lactate/ethanol mixtures, as well as methylene chloride/methanol/H_2O (40/40/20) and ethyl acetate, being the most commonly used solvent for extracting carotenoids for utilization in food products, but not considered to be an environmentally friendly solvent.

With respect to the vegetable sources utilized, Ishida and Chapman[54] determined the major carotenoid contents in several lyophilized vegetable powders, in particular, carrots for the carotene content (particularly β-carotene), white corn for lutein, red tomatoes for the high *trans*-lycopene isomer content and Tangerine tomatoes for the high *tetra-cis*-lycopene isomer content. The distinction between the two isomers is important because *cis*-lycopene isomers are more hydrophilic than the *trans*-isomers.

Additionally, the authors tested the efficacy of two added antioxidants (α-tocopherol and α-lipoic acid) in protecting carotenoids against oxidative degradation during the extraction process with ethyl lactate.

Lyophilized vegetable powders (0.25-1.0 g) were placed in contact with 10 mL of solvent. Three different temperatures (30, 45, and 60ºC) were studied for periods of extraction up to 5 h of incubation using ethyl lactate as solvent. The authors determined that carotenoids underwent degradation over time, in particular lutein. The optimal temperature and extraction time for both lycopene isomers (*trans* and *cis*-isomer) was 45ºC and 1 h. In the case of β-carotene and lutein, they were most efficiently extracted at 60ºC after 0.5 h and at 30ºC after 2 h of extraction time, respectively. The effect of ethanol as cosolvent on carotenoid extraction was also studied using various ethanol + ethyl lactate mixtures (0, 40, 60, 80 and 100% of ethanol) and different extraction temperatures. The addition of ethanol increased the carotenoid extraction yield. When 100% of ethanol was used, the largest amount of lutein was extracted from white corn with incubation times up to 5 h at 30ºC. In the case of β-carotene from carrots, the highest amount was extracted with 100% ethanol at 60ºC after 2 h. In the case of *tetra-cis*-lycopene from Tangerine tomatoes, maximum yields were attained between 60 and 100% ethanol at 60ºC and 1 h of extraction time. The addition of ethanol seems to stabilize this isomer, partially avoiding the thermal degradation. In the case of *trans*-lycopene from red tomato, 100% ethyl lactate is the most efficient solvent at 30ºC, but this behavior changes as the extraction temperature increases. At 60ºC extraction temperature, after 5h extraction time, the highest amount of *trans*-isomer was obtained with 100% ethanol. This behavior was due to the isomerization of *trans*-lycopene into *cis*-lycopene with temperature (*cis*-lycopene isomers are barely detectable in the unheated red tomato powder) and because spectrophotometer was used to measure the carotenoid content in the extracts. Isomerization was produced in both solvents, but in ethanol was greater than in ethyl lactate and the spectrophotometer provides a total concentration of lycopene, but does not distinguish between *cis*- and *trans*-lycopene isomers. It was clear from conducting further experiments and use of HPLC to determine the lycopene isomers. In this sense, red tomato powder was extracted at 60 ºC for 2 h, using 100% ethyl lactate and 100% ethanol. The authors concluded that

the formation of *cis*-isomers is higher in ethanol than in ethyl lactate, but the total concentration of lycopene extracted is lower. The same experiment was carried out with Tangerine tomatoes powder and the greater stability of the *tetra-cis*-lycopene isomer in both ethyl lactate and ethanol was noted as compared with the *trans*-lycopene isomer, and the higher extracted amounts of total lycopene with ethanol than with ethyl lactate. In general, ethyl lactate and ethanol might have a protective effect on the integrity of *tetra-cis*-lycopene.

Additionally, Ishida and Chapman[54] tested the efficacy of two antioxidants, namely α-tocopherol and α-lipoic acid, added at different concentrations (from 0 to 200 mg/10 mL of solvent), in protecting carotenoids against oxidative degradation during the extraction process with ethyl lactate. Carotenoids yields increased with the addition of both antioxidants, indicating their protective effect. In general, both antioxidants and all concentrations added to the sample seemed to give similar protection, although lutein indicated greater stability using lipoic acid.

The extractability of these carotenoids was also compared using several solvents. Extraction with methylene chloride/methanol/water (MMH) (40:40:20), ethyl acetate, ethyl lactate and ethanol as solvents were carried out at 60ºC for 2 h. Ethanol was the most effective solvent for extracting lutein from white corn and β-carotene from carrots (8.38 and 1140.54 $\mu g \cdot g^{-1}$ of dry weight, respectively), whereas MMA was the most effective solvent for extracting *trans*-lycopene from red tomatoes and *cis*-lycopene from Tangerine tomatoes (558.48 and 1817.09 $\mu g \cdot g^{-1}$ of dry weight, respectively). Ethyl lactate extracted between 79-75% of the maximum *trans*-lycopene, β-carotene and lutein achieved and 65% of the maximum *cis*-lycopene. Therefore, MMA is the most efficient solvent, but because of its toxicity, it cannot be used for ingested products and ethyl acetate (which is commonly used to extract carotenoids for food products) is lesser effective than ethyl lactate. Moreover, if red tomato powder is extracted at 45 ºC for 1 h using ethyl lactate, the amount extracted (541 $\mu g \cdot g^{-1}$ of dry weight) is almost equal to that obtained using MMH after 2 h at 60ºC extraction. On the other hand, mixture ethyl lactate/ethanol can be interesting application. For example, red tomato powder can be extracted at 45°C for 2 h using 60% ethanol in ethyl lactate, and the extracted amount goes up to 724 $\mu g \cdot g^{-1}$ of dry weight, which is much larger than that extracted using MMH. Under all these conditions considerable isomerization happened, however this fact could be even more beneficial because there are strong evidence on higher bioavailability of *cis*-lycopene compared to *trans*-lycopene. Therefore, Ishida and Chapman[54] concluded that ethyl lactate could be a good solvent for extracting carotenoids, particularly, lycopene and could replace toxic petroleum-derived solvents, such as methylene chloride, hexane and acetone which have been patented for extracting carotenoids from food.[101-103]

20.4.3.3.2 Extraction of lycopene from tomato waste

Strati and Oreopoulou[55] studied the capability of different organic solvents (hexane, acetone, ethanol, ethyl acetate and ethyl lactate) to extract carotenoids from tomato waste, composed by dry skin and seeds. In industry, tomato wastes generally accounting for 10 to 40% of the total tomato processed for tomato products.[104]

The tomato waste/solvent ratio was 1:10 and different extraction parameters (type of solvent, extraction time, temperature, and number of successive extractions) were tested. Regarding the extraction temperature, the experiments were carried out at 25ºC. Carotenoid concentration (expressed as lycopene, since this carotenoid represents around 80-90% of total tomato carotenoids) increased with time and the equilibrium concentration was achieved at approximately 30 min of extraction time. The concentration was considerably low in ethanol (0.38 mg/L) and higher in ethyl lactate (12.52 mg/L) at equilibrium. The concentration for the other solvents was between 1.99-2.82 mg/L.

Based on these results, 30 min was chosen by the authors for studying the effect of successive extractions steps on the recovery of carotenoids, which was expressed as percentage of the total amount extracted from three successive extraction steps. That is, after an extraction, the residue was put again in contact with the same quantity of solvent, up to a total of three times. Three different temperatures (25, 50, and 70ºC) were tested, except for hexane (25, 50, and 60ºC) and acetone (25 and 50ºC) due to their lower boiling points. Carotenoid recovery was significantly affected by the number of extractions regardless of the solvent and temperature applied. The recovery of carotenoids using medium or nonpolar solvents (ethyl acetate and hexane) in the first cycle was higher than that obtained with more polar solvents (acetone, ethanol and ethyl lactate). In particular, 70.1-73.4% and 71.4-74.4% of total extracted carotenoids was recovered in the first cycle with hexane and ethyl acetate, respectively. In the case of ethyl lactate, the carotenoids recovered in the first cycle ranged between 57.5% and 67.0%. In all cases, the first extraction step is the controlling one and carotenoids recovered in the third cycle were lower than 16%.

With regard to the effect of temperature and type of solvent on carotenoids extraction yield (mg carotenoid per kg of dry tomato waste), the increase in extraction temperature generally increased the extraction yield. Nevertheless, there were no significant differences at 25 or 50ºC in the case of hexane and ethyl lactate extracts. However, close to the highest temperature studied a greater extractability of carotenoids was observed for all solvents.

Ethyl lactate presented remarkably higher yields at the three extraction temperatures (202.73 mg/kg at 25ºC and 243.0 mg/kg at 70ºC), followed by acetone (13.7% at 25ºC and 21.4% at 50ºC of the maximum yield obtained with ethyl lactate). Ethanol showed the lowest yield (<10% of the maximum yield obtained with ethyl lactate, at 70ºC). Therefore, 30 min extraction time at 70ºC, using several extraction steps with ethyl lactate solvent proved to be a more advantageous method than employing ethyl acetate, which is the current solvent employed to extract carotenoids for food applications.

20.4.3.3.3 Extraction of astaxanthin from Xanthophyllomyces dendrorhous

Astaxanthin is a keto-carotenoid pigment, belonging to xanthophylls group. This pigment confers the coloration to some birds, crustaceans and salmons and is used in food, cosmetic, and pharmaceutical application due to its antioxidant activity.[106] Wu *et al*.[105] developed an environmentally friendly extraction method to extract astaxanthin from the red yeast *Xanthophyllomyces dendrorhous* using green solvents.

First of all, the most efficient acid and organic solvent in disrupting the yeast cell wall and extracting astaxanthin were selected. Four acids were checked: hydrochloric,

acetic, citric, and lactic acid. The last one was the most efficient acid. In the case of organic extraction solvent, petroleum ether, acetone, ethanol, hexane, and ethyl lactate were tested. Ethyl lactate was the most efficient solvent and acetone and ethanol were 85 and 82% as efficient as ethyl lactate, respectively, while petroleum ether and hexane showed poor extraction efficiency. In the view of these results, ethyl lactate and ethanol were used as extraction solvents and lactic acid was applied to disrupt the yeast cell to enhance the extractability of astaxanthin.

Lyophilized powder (0.2 g) was put in contact with 10 mL of 5 $mol \cdot L^{-1}$ lactic acid at controlled temperature. After cell disruption, the mixture was centrifuged and the liquid phase removed. Then the fraction of disrupted cells was extracted with 15 mL of organic solvent at room temperature. The process parameters were optimized to estimate the best conditions for the extraction yield of astaxanthin and for that, a four-factor three-level orthogonal array design was used. The factors and levels were the following: disrupting temperature (35, 50, and 65ºC), disrupting time (10, 60, and 110 min), type of solvent (ethyl lactate and ethyl lactate + ethanol in 1/1 and 3/1 ratios) and extraction time (10, 30, and 50 min).

Disrupting temperature, disrupting time and type of extraction solvent have significant influence on astaxanthin yield. Extraction time was the most important parameter although a long extraction time may lead to oxidation of astaxanthin. For this reason, the efficacy of two antioxidant, namely ascorbic acid and α-tocopherol, was checked in protecting against oxidative degradation during the extraction. Authors concluded that the optimal conditions for the extraction of astaxanthin were 30 min of extraction time, 65ºC of disrupting temperature, 60 min of disrupting time, and ratio 1/1 of the ethyl lactate + ethanol solvent.

In regard to the addition of antioxidants, ascorbic acid was tested to protect astaxanthin during 6 h of cell wall disruption step and using lactic acid at 65ºC. Concentrations of ascorbic acid ranged from 0 to 18 mg per mL of lactic acid. Ascorbic acid prevented loss of astaxanthin, but its effect was relatively modest. Additionally, α-tocopherol was used during the extraction step with 100% ethyl lactate. As with ascorbic acid, concentrations ranged from 0 to 18 mg per mL of ethyl lactate and the studied action time was 6 h. With 12 mg of α-tocopherol, maximal amounts of astaxanthin were extracted after 1 h extraction time (α-tocopherol caused an increase of 11% in astaxanthin yield). Quantities above 12 mg did not seem to be effective.

Finally, different extraction methods with conventional solvents for astaxanthin extraction were compared with the new method to corroborate its efficiency. In this sense, DMSO (for disrupting the cell wall) + acetone (the extraction solvent) were compared with the use of lactic acid + ethyl lactate/ethanol solvent (1/1 ratio) + α-tocopherol. The astaxanthin yields obtained were very similar (1484.85 μg/g using DMSO and 1464.16 μg/g using the ethanol/ethyl lactate solvent). Similar results were obtained using other conventional solvents, demonstrating that the method developed could replace the traditional astaxanthin extraction using toxic or flammable solvents.

20.4.3.4 Extraction of γ-linolenic acid from Arthrospira platensis

γ-linolenic acid (GLnA) is a ω-6-polyunsaturated C18:3 fatty acid which is the first intermediate product in the conversion of linoleic acid into arachidonic acid.[107] This fatty acid is found in relatively high abundance in the lipids of several plant seed oils, and nowadays, the most used source of this acid is borage oil.[108] The amount of GLnA in *Arthrospira platensis* (*Spirulina microalgae*) ranges from 18 to 21 wt% of the total fatty acids in the lipids. Furthermore, *Spirulina* shows several advantages in terms of a large scale commercial cultivation so it is presented as an alternative source of GLnA.[109]

Golmakani *et al*.[60] evaluated the use of two green extraction methods, that is, gas expanded liquids (GXLs) using CO_2 as condensable gas and pressurized liquid extraction (PLE), and two green solvents, namely ethyl lactate and ethanol, to obtain γ-linolenic acid-enriched fractions from *Arthrospira platensis*. Response surface methodology (RSM) concerning central composite design was employed for statistical design of PLE. A four-factorial (extraction temperature, extraction pressure, extraction time and solvent composition) and three-level central composite design was performed. In the case of GXLs extractions, authors used Taguchi's L_9 (3^4) orthogonal array with four factors at three levels. The factors considered were extraction temperature, extraction pressure, extraction time, and the fraction of ethanol in CO_2. In the case of PLE, experimental temperature values ranged from 60 to 180ºC, pressures from 3.4 to 20.7, extraction times from 5 to 15 min (one cycle only) and solvent composition from 100% ethanol to 100% ethyl lactate. In the case of GXLs, temperatures ranged from 40 to 80ºC, pressures from 10 to 30 MPa, extraction times from 30 to 90 min and ethanol percentage from 10 to 50 wt%.

In the case of PLE, the maximum overall extraction yield (mass extract/mass *Spirulina*) was 22.7%, the concentration of lipids in the extracts was 47.9 wt%, the concentration of GLnA was 11.4 wt% and the GLnA recovery was 74.7%. In the case of GXLs extractions, the maximum overall yield achieved was 7.4%, the maximum percentage of lipids and GLnA in the extracts were, respectively, 48.0 and 11.7 wt%, and the maximum recovery of GLnA was 35.3%. In view of the results, the total yield and the recovery of GLnA was higher in PLE than GXLs while percentage of lipids and GLnA in the extracts was similar for both extraction processes at the studied conditions. Therefore, the higher yields obtained in PLE resulted in higher recoveries of GLnA when using this extraction technique.

The authors defined extraction temperature as the most significant factor in PLE extractions.[60] Temperature has a positive effect on extraction yield, because when temperature was increased the extraction yield was higher, and has the opposite effect on GLnA concentration in the extract. On the other hand, pressure has a small effect on the extraction yield and it was not considered as a significant factor in any of the responses studied. In the case of GXLs, temperature was the less significant factor, whereas extraction time and fraction of ethanol (wt%) were the main factors. Increasing extraction time and ethanol fraction in CO_2, total extraction yield also increased, unlike supercritical fluid extractions (SFE) in which, temperature and pressure play major roles on the solubility of the compounds.[60] With SFE, higher GLnA and lipid purities can be achieved, but the lower yield gives lower recoveries of GLnA.

The optimal extraction conditions according to Golmakani *et al.*[60] study were as follows: 180ºC, 207 bar, 15 min and ethanol + ethyl lactate (50:50 v/v) in the case of PLE, predicting 21.5% of total yield and 63.9% of GLnA recovery. For GXLs extraction optimal extraction conditions were 40ºC, 300 bar, 90 min and CO_2/ethanol with 50% ethanol, and the predicted results were 7.0% of total yield and 28.0% of GLnA recovery. These results were experimentally corroborated (at least three times) by the authors and the prediction was pretty close to the mean real extraction process. Therefore, different extraction alternatives using ethyl lactate as alternative solvent were successfully applied to produce enriched fractions in γ-linolenic acid.

20.4.3.5 Extraction of thymol from *Thymus genus*

Villanueva *et al.*[62] reported a comparison between the performance of different green solvents for extracting thymol from different thyme plants. The genus *Thymus* (*Lamiaceae* family) is an aromatic plant very rich in essential oil compounds, which are the most valued constituents of the herb. Thyme essential oil is formed by terpenes and its oxygenated derivative compounds (alcohols, aldehydes, ketones, etc.) and is appreciated for food flavoring, in cosmetic, perfumery and in the pharmaceutical industry.

Thymol (2-isopropyl-5-methylphenol) is the main monoterpene phenol, isomeric with carvacrol, found in thyme essential oil. This compound has revealed several biological properties, such as antibacterial, antifungal, anti-inflammatory, antioxidant, and immunomodulatory.[110,111]

Villanueva *et al.*[62] studied the pressurized liquid extraction (PLE) of thyme varieties (*Thymus vulgaris*, *Thymus zygis* and *Thymus citriodorus*) using ethanol, limonene, and ethyl lactate solvents, at different extraction temperatures (60, 130, and 200ºC). Supercritical fluid extraction with pure CO_2 (SFE-CO_2)[116] and with the three green solvents used as cosolvent were also tested. Additionally, the authors reported solubility data of thymol in limonene and ethanol at ambient pressure and temperatures in the range 30-43ºC and the results were compared with ethyl lactate solubility data previously reported.[81] In this respect, it was observed that thymol is very soluble in the three solvents, particularly in ethanol, followed by ethyl lactate, with concentrations around 90 wt% at the highest studied temperature. On the contrary, the solubility of thymol in limonene is somewhat lower (~73 wt%).

In regard to PLE extractions, the highest concentration of thymol in the *T. vulgaris* extracts was obtained with limonene at 60ºC (see Table 20.4.23). Limonene also produced the highest concentrations of thymol. Therefore, although solubility of thymol in limonene is lower, the selectivity of limonene to extract thymol from thyme leaves was larger in comparison with the other two solvents.

Table 20.4.23. Thymol concentration (g/g extract x100) obtained in the PLE of *Thymus vulgaris*.

	60ºC	130ºC	200ºC
Ethyl lactate	13.88 ± 0.10	11.78 ± 0.43	4.65 ± 0.02
Ethanol	6.59 ± 0.89	6.89 ± 0.78	4.88 ± 0.67
Limonene	18.15 ± 0.25	12.59 ± 0.49	10.06 ± 0.82

Regarding thymol yield (mg of thymol extracted/g of thyme plant) (Table 20.4.24) at 200ºC it was higher than those obtained using traditional extraction methods, such as Soxhlet with hexane or steam distillation, and was higher than those obtained using subcritical water extraction. Moreover, the thymol yield was just slightly lower than those obtained by PLE extraction with non-green solvents like hexane, dichloromethane or ethyl acetate.[112,113]

Table 20.4.24. Thymol yield (mg/g plant) obtained by PLE[62,112,113] and by conventional methods.[112,113]

Pressurized Liquid Extraction (PLE)							Conventional methods	
Limonene	Ethyl lactate	Ethanol	Water	Hexane	Dichloro methane	Ethyl acetate	Soxlet hexane	Steam distillation
9.5	10.5	10.6	7.0	10.7	12.2	12.8	6.8	8.2

In the case of the other two studied thyme species, although the total extraction yields obtained from *T. zygis* were higher than those attained from *T. vulgaris* at the same temperature (60ºC), the thymol concentrations were lower, presuming a higher content of thymol in *T. vulgaris* in comparison with *T. zygis*. In the case of *T. citriodorus*, thymol was detected, but could not be quantified despite the solvent employed, probably due to lower thymol content in this thyme variety.

With respect to the SFE extractions with pure CO_2 (SFE-CO_2), the total extraction yield increases when higher extraction pressures were applied.[114] As in the case of PLE, the highest thymol yield were obtained from *T. vulgaris* extractions[114] (10.27 mg thymol/g plant at 15 MPa, 40ºC and 35 kg CO_2/kg thyme ratio) and for this thyme species, the increase of CO_2 flow resulted in higher thymol yields than those produced by increasing extraction pressure. On the other hand, similar thymol yields were obtained in the case of *T. zygis* and *T. citriodorus* despite of the extraction pressure or CO_2 flow employed, indicating that thymol would be exhausted from plant matrix. Additionally, the SFE of *T. vulgaris* was also studied using the three green liquid solvents as CO_2 cosolvents.[62] Three extractions were carried out at 15 MPa and 40ºC (best conditions assessed with pure CO_2) and concentrations of 10 wt% of ethyl lactate, ethanol, or limonene in CO_2. Yet, no

improvement of thymol extraction was obtained, with thymol yields in the range of 7-9 mg/g.

Villanueva *et al*.[62] concluded that any of the three green liquid solvents studied, namely ethanol, ethyl lactate and limonene, can efficiently extract thymol from thyme plants, with limonene being the solvent that produced the highest concentrations, due to its lipophilic character. Although PLE produced similar thymol recovery than SFE, considerably higher concentrations of thymol were obtained by SFE-CO_2 (up to 31.0 wt%) supporting the selective extraction of volatile oil compounds from plants and herbs using SFE-CO_2.

4. FINAL REMARKS

The increasing environmental regulations and social consciousness regarding ecological problems is promoting the development of the Green Chemistry, seeking for eco-friendly chemical processes, and tending to replace petroleum-derived solvents (most of them toxic, flammable, and/or corrosive) by agrochemical solvents. Ethyl lactate is one of these agrochemical 'green' solvents, and can be derived from processing of carbohydrate feedstocks, such as wastes from corn or sugar crops. Several processes are being studied and patented in order to reduce its production cost and to promote its use as a replacement of non-green solvents.

Ethyl lactate is fully biodegradable (breaks down into carbon dioxide and water), is non-corrosive, non-carcinogenic, non-teratogenic and non-ozone depleting. Ethyl lactate has a high boiling point, low vapor pressure and low surface tension. It is remarkable that its molecular structure possesses a specific topology, allowing the ability to form intra- and inter-molecular association *via* hydrogen bonds, either as proton donor or proton acceptor. Thus, ethyl lactate presents high solvent power since it can cover a wide range of polarities. It was affirmed as GRAS and approved by the U.S. FDA as pharmaceutical and food additive.

Along with other lactate esters, ethyl lactate is used in the coating industry (wood, polystyrene, metals, etc.) and as a cleaning agent dissolving pesticides, polyurethane resins, metal surfaces, etc., and can successfully remove oils, paint, dried ink, adhesives and solid fuels. In this respect, ethyl lactate is replacing some traditional solvents such as butan-2-one (MEK), 4-methylpentan-2-one (MIBK), acetone, toluene and xylenes. Regarding other uses in the chemical industry, ethyl lactate is being currently explored as green reaction medium for chemical synthesis, for example for the production of 1,2-propanediol. Moreover, ethyl lactate production and subsequent hydrolysis can be used to obtain high-purity lactic acid.

Ethyl lactate is currently applied in both pharmaceutical and cosmetic industry, since it can improve the solubility of many classes of bioactive compounds (antihistamines, antivirals, antibiotics, etc.) and can favor the topical penetration of cosmetic and pharmaceutical principles into skin. Thus, ethyl lactate is used in creams, powders, masks, serums, pastes, sprays, lotions and other formulas.

With respect to the food business, it has to be pointed out that ethyl lactate is a flavor compound found naturally in small quantities in a wide variety of foods such as meat, some fruits, soy products and fermented foods, such as wine or beer. Thus, ethyl lactate is

well accepted as ecological solvent for food processing. Several potential applications are related to the extraction of carotenoids from different plant matrix, such us tomatoes, carrots, white corn and microalgae, and the recovery of other high value bioactive substances from plants and herbs.

Particularly, ethyl lactate is a potential novel solvent for the removal of caffeine from green coffee beans, demonstrating high decaffeination power and low alteration of the phenolic and lipid compounds content of the beans. Furthermore, ethyl lactate is being studied for the decaffeination of other vegetal matter, such as green tea leaves.

Another important application of ethyl lactate is related with the edible oil industry, taking advantage of the partial liquid-liquid miscibility that present the mixtures of ethyl lactate with different lipid type substances. This property could be exploited to develop new separation processes, similar to those mentioned in this chapter, namely the recovery of squalene from olive oil deodorized distillates and the extraction of tocopherols from olive oil. In both applications, the yield and separation factors obtained indicate good selectivity of using ethyl lactate as an extractive solvent, and demonstrate the viability of developing liquid-liquid countercurrent process using green ethyl lactate solvent in edible oil industrial applications.

Finally, it should be stated that ethyl lactate is a novel ecofriendly solvent for potential applications in supercritical fluid technology, as a cosolvent of carbon dioxide. Ethyl lactate can be readily dissolved in CO_2 in the amounts usually employed for supercritical extraction processes (15-20 wt% or lower). Additionally, at the typical temperatures employed (35-70^{o}C) the ethyl lactate + CO_2 system presents homogeneous (single) phase at relative low pressures.

Furthermore, since CO_2 exhibits high solubility in ethyl lactate (higher than in ethanol) it can be employed to promote different approaches in the perspective of expanded liquid solvents, such as high pressure chemical reactions and/or anti-solvent precipitation processes. By dissolving CO_2 in ethyl lactate at high pressure, the liquid solvent properties (viscosity, surface tension, diffusion rates, solubility of reagents, catalysts and substrates, density, and polarities) can be modified. In this respect, ethyl lactate is a good candidate to form gas expanded liquid solvents, with the additional advantage of being itself a green solvent.

APPENDIX A:

GC-EoS modeling of ethyl lactate vapor pressure

The Group Contribution Equation of State (GC-EoS) has two contributions to the residual Helmholtz energy of the system: a repulsive hard sphere Carnahan-Starling type term and an attractive term, which combines the group contribution approach with the local-composition mixing rules. A complete explanation of the model is given by Skjold-Jørgensen.[18]

The model parameters are the following: the critical hard sphere diameter (d_c) which is characteristic of each substance, five pure-group parameters (T*, q, g*, g' and g") and four binary interaction parameters (k_{ij}*, k_{ij}', α_{ij} and α_{ji}). The reference temperature (T*) and group surface area (q) are not adjustable parameters while the pure group parameters (g*, g' and g'') are usually regressed using adequate vapor pressure data.

Figure 20.4.19 shows the chemical structure of ethyl lactate. According to the current GC-EoS parameter table,[19] the group composition of ethyl lactate molecule can be described by means of two CH_3, one CH_2COO and one CHOH groups (Set 1). Nevertheless, considering that ethyl lactate is the ester of lactic acid, a more appropriate representation should include the definition of a new alcohol-ester functional group (CHOHCOO) (see Figure 20.4.19); thus the ethyl lactate molecule can be represented by two CH_3, one CH_2 groups, and the new CHOHCOO group (Set 2). The pure group and group interaction parameters were reviewed by Villanueva *et al.*[17] and are summarized in Tables 20.4.25 and 20.4.26 The critical temperature and pressure of ethyl lactate are T_c = 506.01K, p_c = 28.22 bar, as reported by Villanueva *et al.*[17]

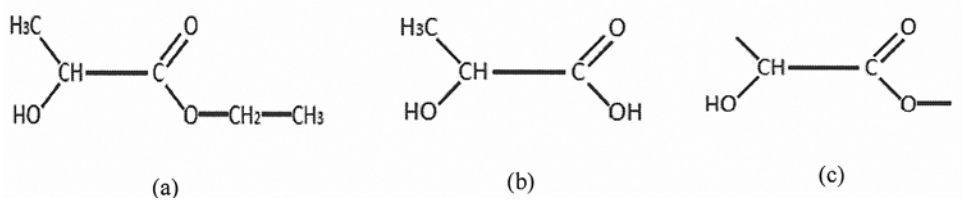


Figure 20.4.19. Chemical structure of (a) ethyl lactate, (b) lactic acid and (c) the alcohol-ester group.

Table 20.4.25. GC-EoS pure group parameters.

	Ref. temp.	Group surf. area	Pure group energy parameters		
	T*	q	g	g'	g''
CH_3	600	0.848	316910	–0.9274	0.0
CH_2	600	0.540	356080	–0.8755	0.0
$COOCH_2$	600	1.420	831400	–1.0930	0.0
OHCH	512.6	0.908	1207500	–0.6444	0.0
CHOHCOO	600	1.788	891900	–1.0993	0.0

Table 20.4.26 GC-EoS binary group interaction parameters.

i	j	Attractive energy parameters		Non-randomness parameters	
		k_{ij}	k'_{ij}	α_{ij}	α_{ji}
CHOHCOO	CH_3/CH_2	1.054	0.018	1.777	1.777
$COOCH_2$	CH_3	0.869	0.0	0.0	0.0
	CHOH	0.996	–0.163	0.654	–2.612
OHCH	CH_3	0.715	0.0	10.220	1.471

APPENDIX B:

Thermodynamic UNIFAC modeling of the solubility of phenolic acids in ethyl lactate

Model equations correspond to the modified UNIFAC (Dortmund) model.[91] The activity coefficient of solute (γ_i) is calculated considering a combinatorial (γ_i^C) and a residual (γ_i^R) contributions:

$$\ln\gamma_i = \ln\gamma_i^C + \ln\gamma_i^R$$

The combinatorial part is given by:

$$\ln\gamma_i^C = 1 - V'_i + \ln V'_i - 5q_i\left[1 - \frac{V_i}{F_i} + \ln\left(\frac{V_1}{F_i}\right)\right]$$

where:

$$V'_i = \frac{r_i^{3/4}}{\sum x_j r_j^{3/4}},\ V_i = \frac{r_i}{\sum x_i r_i},\ r_i = \sum \upsilon_k^{(i)} R_k,\ F_i = \frac{q_i}{\sum x_i q_i},\ q_i = \sum \upsilon_k^{(i)} Q_k$$

The residual part is calculated according to the following equation:

$$\ln\gamma_i^R = \sum \upsilon_k^{(i)} (\ln\Gamma_k - \ln\Gamma_k^{(i)})$$

where:

$$\ln\Gamma_k = Q_k\left(1 - \ln\left(\sum Q_m \psi_{mk}\right) - \sum \frac{\theta_m \psi_{km}}{\sum \theta_n \psi_{nm}}\right)$$

$$\theta_m = \frac{Q_m X_m}{\sum Q_n X_n},\ X_m = \frac{\sum v_m^{(i)} x_j}{\sum\sum v_n^{(i)} x_j},\ \psi_{nm} = \exp\left(-\frac{a_{nm} + b_{nm}T + c_{nm}T^2}{T}\right)$$

The physical properties of pure phenolic acids were reported by Manic *et al.*[81] and are given in Table 20.4.27. The group compositions of phenolic acids and ethyl lactate are given in Table 20.4.28.

Group interaction parameters were those reported Gmehling *et al.*,[91] except for the ACOH-COOH interaction, which were reviewed by Manic *et al.*[81] All group interaction parameters employed for solubility calculation are summarized in Table 20.4.29.

Table 20.4.27. Physical properties of phenolic acids.

Compound	T_m/K	ΔH_{fus}/kJmol^{-1}	ΔC_p/Jmol^{-1}K^{-1}
vanillic acid	480.7	29.1	64.4
ferulic acid	444.9	31.9	73.7
caffeic acid	464.1	39.85	162.7

Table 20.4.28. Group composition of phenolic acids and ethyl lactate.

UNIFAC main group	Subgroup k	R_k group van der Waals volume	Q_k group van der Waals surface area	Compound group composition			
				caffeic acid	vanillic acid	ferulic acid	ethyl lactate
1. CH_2	CH_3	0.6325	1.0608				2
	CH_2	0.6325	0.7081				1
2. CH=CH	CH=CH	1.2832	1.2489	1		1	
3. ACH	AC	0.3763	0.2113	1	2	2	
	ACH	0.3763	0.4321	2	3	3	
4. $ACCH_2$	$ACCH_3$	0.9100	0.9490				
5. OH	OH(s)	1.0630	0.8663				1
8. ACOH	ACOH	1.0800	0.9750	2	1	1	
11. CCOO	CHCOO	1.2700	1.4228				1
13. CH_2O	OCH_3	1.1434	1.6022		1	1	
20. COOH	COOH	0.8000	0.9215	1	1	1	

Table 20.4.29. UNIFAC interaction parameters between main groups of Table 20.4.28.

n	m	a_{nm}	b_{nm}	c_{nm}	a_{mn}	b_{mn}	c_{mn}
1. CH_2	2. CH=CH	189.66	-0.2723	0.0	-95.418	$0.6171 \cdot 10^{-1}$	0.0
	3. ACH	114.20	$0.9330 \cdot 10^{-1}$	0.0	16.070	-0.2998	0.0
	4. $ACCH_2$	7.3390	-0.4538	0.0	47.200	0.3575	0.0
	5. OH	2777.0	-4.6740	$0.1551 \cdot 10^{-2}$	1606.0	-4.7460	$0.9181 \cdot 10^{-3}$
	8. ACOH	1381.0	-0.9977	0.0	1987.0	-4.6150	0.0
	11. CCOO	98.656	1.9294	$-0.3133 \cdot 10^{-2}$	632.22	-3.3912	$0.3928 \cdot 10^{-2}$
	13. CH_2O	233.10	-0.3155	0.0	-9.6540	$-0.3242 \cdot 10^{-1}$	0.0
	20. COOH	1182.2	-3.2647	$0.9198 \cdot 10^{-2}$	2017.7	-9.0933	$0.1024 \cdot 10^{-1}$
3. ACH	4. $ACCH_2$	139.20	-0.6500	0.0	-45.330	0.4223	0.0
	5. OH	3972.0	-13.160	$0.1208 \cdot 10^{-1}$	3049.0	-12.770	$0.1435 \cdot 10^{-1}$
	8. ACOH	1356.0	-2.1180	0.0	2340.0	-5.0430	0.0
	11. CCOO	-274.54	0.9149	0.0	622.73	-1.7605	0.0
	13. CH_2O	-87.080	-0.1859	0.0	179.00	$0.5615 \cdot 10^{-1}$	0.0
	20. COOH	69.561	1.8881	0.0	613.32	-1.5950	0.0
5. OH	8. ACOH	83.910	-1.2620	0.0	465.40	-1.8410	0.0
	11. CCOO	973.80	-5.6330	$0.7690 \cdot 10^{-2}$	310.40	1.5380	$-0.4885 \cdot 10^{-2}$
	13. CH_2O	1102.0	-7.1760	$0.9698 \cdot 10^{-2}$	1631.0	-7.3620	$0.1176 \cdot 10^{-1}$
	20. COOH	-1295.0	4.3634	0.0	1525.8	-4.9155	0.0
8. ACOH	11. CCOO	-212.90	0.0	0.0	-224.40	0.0	0.0
	13. CH_2O	-329.30	0.0	0.0	-80.580	0.0	0.0
	20. COOH	415.72	-1.97	0.0	120.50	-2.37	0.0
11. CCOO	13. CH_2O	195.30	-9.75	$0.4051 \cdot 10^{-1}$	824.20	-6.0090	$0.8271 \cdot 10^{-2}$
	20. COOH	62.031	1.0567	0.0	59.594	-0.7120	0.0
13. CH_2O	20. COOH	521.48	0.0	0.0	-310.82	0.0	0.0

REFERENCES

1 R.A. Sheldon, *Green Chem*., **7**, 267 (2005).
2 P. Anastas, and N. Eghbali, *Chem. Soc. Rev.*, **39**, 301 (2010).
3 E. Lynge, A. Anttila, and K. Hemminki, *Cancer Causes Control*, **8**, 406 (1997).
4 R.K. Khandal, S. Kaushik, G. Seshadri, and D. Khandal, **Handbook of detergents Part F: Production**, *CRC Press*, Florida, 2009.
5 S. Kromusa, B. Wachterc, W. Koschuha, M. Mandlc, C. Krotschecka, and M. Narodoslawsky, *Chem. Biochem. Eng. Q.*, **18**, 7 (2004).
6 C.S.M. Pereira, V.M.T.M. Silva, and A.E. Rodrigues, *Green Chem*., **13**, 2658 (2011).
7 S. Aparicio, and R. Alcalde, *Green Chem*., **11**, 65 (2009).
8 S. Aparicio, *J. Phys. Chem. A*, **111**, 4671 (2007).
9 S. Aparicio, S. Halajian, R. Alcalde, B. García, and J.M. Leal, *Chem. Phys. Lett.*, **454**, 49 (2008).
10 N. Borho, and M.A. Suhm, *Org. Biomol. Chem*., **1**, 4351 (2003).
11 J-T. Chen, and H-P. Chu, *J. Chem. Eng*., **52**, 650 (2007).
12 J.A. Riddick, W.B. Bunger, and T.K. Sakano, **Organic solvents: physical properties and methods of purifications** 4th edition (vol. 2), *Wiley-Interscience*, New York, 1986.
13 S. Peña-Tejedor, R. Murga, M.T. Sanz, and S. Beltrán, *Fluid Phase Equilib.*, **230**, 197 (2005).
14 J.M. Resa, J.M. Goenaga, and A.I. Sánchez-Ruiz, *J. Chem. Eng.*, **51**, 1294 (2006).
15 F.D. Snell, and C.T. Snell, **Dictionary of commercial chemicals** 3rd ed., *D. Van Nostrand Co*., New Jersey, 1962.
16 M. Inal, **Ethyl lactate production by hybrid processes: determination of phase diagrams and evaluation of performance of organophilic pervaporation membranes**, Master of Science Thesis, *Middle East Technical University*, Turkey, 2003.
17 D. Villanueva Bermejo, E. Ibáñez, R.P. Stateva, and T. Fornari, *J. Chem. Eng*., **58**, 301 (2013).
18 S. Skjold-Jørgensen, *Fluid Phase Equilib*., **16**, 317 (1984).
19 T. Fornari, *Fluid Phase Equilib*., **262**, 187 (2007).
20 R. Datta, and M. Henry, *J. Chem. Technol. Biotechnol*., **81**, 1119 (2006).
21 P. Delgado, M.T. Sanz, and S. Beltrán, *Chem. Eng. J*., **126**, 111 (2007).
22 S. Dassy, H. Wiame, and F.C. Thyrion, *J. Chem. Technol. Biotechnol*., **59**, 149 (1994).
23 Q. Yixin, P. Shaojun, W. Shui, Z. Zhiqiang, and W. Jidong, *Chin. J. Chem. Eng*., **17**, 773 (2009).
24 M.T. Sanz, R. Murga, S. Beltran, J.L. Cabezas, and J. Coca, *Ind. Eng. Chem. Res*., **43**, 2049 (2004).
25 E.M. Filachione, E.J. Costello, and C.H. Fisher, *J. Am. Chem. Soc*., **73**, 5265 (1951).
26 Y. Halpern. US Patent 6,583,310.
27 H. Kwak, D.W. Hwang, Y.K. Hwang, and J-S. Chang, *Sep. Purif. Technol*., **93**, 25 (2012).
28 D. Bai, Z. Yan, Q. Wei, X. Zhao, X. Li, and S. Xu, *Biochem. Eng. J*., **19**, 47 (2004).
29 P. Kasinathan, H. Kwak, U. Lee, D.W. Hwang, Y.K. Hwang, and J-S. Chang, *Sep. Purif. Technol.*, **76**, 1 (2010).
30 P. Kasinathan, D.W. Hwang, U. Lee, Y.K. Hwang, and J-S. Chang, *Chem. Eng. Sci*., **66**, 4549 (2011).
31 C.H. Holten, **Lactic acid: properties and chemistry of lactic acid and derivatives**, *Verlag Chemie*, Weinheim, 1971.
32 D.T. Vu, A.K. Kolah, N.S. Asthana, L. Peereboom, C.T. Lira, and D. J. Miller, *Fluid Phase Equilib*., **236**, 125 (2005).
33 R. Montgomery, *J. Am. Chem. Soc*., **74**, 1466 (1952).
34 C.S.M. Pereira, S.P. Pinho, V.M.T.M. Silva, and A.E. Rodrigues, *Ind. Eng. Chem. Res.*, 47, 1453 (2008).
35 B. Major, G. Németh, K. Bélafi-Bakó, and L. Gubicza, *Chem. Pap*., **64**, 261 (2010).
36 N. Asthana, A. Kolah, D.T. Vu, C.T. Lira, and D.J. Miller, *Org. Process Res. Dev.*, **9**, 599 (2005).
37 N.S. Asthana, A.K. Kolah, D.T. Vu, C.T. Lira, and D.J. Miller, *Ind. Eng. Chem. Res*., **45**, 5251 (2006).
38 K. Tanaka, R. Yoshikawa, C. Ying, H. Kita, and K. Okamoto, *Chem. Eng. Sci.*, **57**, 1577 (2002).
39 Y. Zhang, L. Ma, and J. Yang, *React. Funct. Polym*., **61**, 101 (2004).
40 D.J. Benedict, S.J. Parulekar, and S-P. Tsai, *Ind. Eng. Chem. Res*., **42**, 2282 (2003).
41 R.A. Troupe, and E. Dimilla, *Ind. Eng. Chem*., **49**, 847 (1957).
42 D. J. Benedict, S.J. Parulekar, and S-P. Tsai, *J. Membr. Sci.*, **281**, 435 (2006).
43 P. Delgado, M.T. Sanz, S. Beltrán, and L.A. Núñez, *Chem. Eng. J*., **165**, 693 (2010).
44 J.J. Jafar, P.M. Budd, and R. Hughes, *J. Membr. Sci*., **199**, 117 (2002).
45 P.M. Budd, N.M.P.S. Ricardo, J.J. Jafar, B. Stephenson, and R. Hughes, *Ind. Eng. Chem. Res*., **43**, 1863 (2004).
46 C.S.M. Pereira, V.M.T.M. Silva, S.P. Pinho, and A.E. Rodrigues, *J. Membr. Sci.*, **361**, 43 (2010).

47 J. Gao, X.M. Zhao, L.Y. Zhou, and Z.H. Huang, *Chem. Eng. Res. Des*., **85**, 525 (2007).
48 C.S.M. Pereira, M. Zabka, V.M.T.M. Silva, and A.E. Rodrigues, *Chem. Eng. Sci*., **64**, 3301 (2009).
49 V.M.T.M. Silva, C.S.M. Pereira, and A.E. Rodrigues, *AIChE J*., **57**, 1840 (2010).
50 M.A. Wicki, and K.E. Nielsen, **US Patent 0,143,599**.
51 T.H.T Vu, H.T. Au, T.H.T. Nguyen, T.T.T. Nguyen, M.H. Do, N.Q. Bui, and N. Essayem, *Catal. Lett*., **143**, 950 (2013).
52 N. Essayem, T.T.H. Vu, T.T.H. Nguyen, T.T.T. Nguyen, and G. Sapaly. **WO Patent 2011/107712**.
53 Z. Findrik, G. Németh, L. Gubicza, K. Bélafi-Bakó, and D. Vasic-Racki, *Bioprocess Biosyst. Eng*., **35**, 625 (2012).
54 B.K. Ishida, and M.H. Chapman, *J. Agric. Food Chem*., **57**, 1051 (2009).
55 I.F. Strati, and V. Oreopoulou, *Int. J. Food Sci.Technol*., **46**, 23 (2011).
56 D. Villanueva Bermejo, P. Luna, M.S. Manic, V. Najdanovic-Visak, G. Reglero, and T. Fornari, *Food Bioprod. Process.*, **92**, 303 (2013).
57 E. J. Hernández, P. Luna, R.P. Stateva, V. Najdanovic-Visak, G. Reglero, and T. Fornari, *J. Chem. Eng*., **56**, 2148 (2011).
58 G. Vicente, A. Paiva, T. Fornari, and V. Najdanovic-Visak, *Chem. Eng*. J., **172**, 879 (2011).
59 X.C. Tombokan, R.M. Aguda, D.A. Danehower, P.K. Kilpatrick, and R.G. Carbonell, *J. Supercrit. Fluids*, **45**, 146 (2008).
60 M. Golmakani, J.A. Mendiola, K. Rezaei, and E. Ibáñez, *J. Supercrit. Fluids*, **62**, 109 (2012).
61 C. Abbas, A. M. Rammelsberg, and K. Beery. **US Patent 0,235,633**.
62 D. Villanueva Bermejo, I. Angelov, G. Vicente, R.P. Stateva, M. Rodríguez García-Risco, G. Reglero, E. Ibáñez, and T. Fornari, *J. Food Eng*. (sent).
63 S.M. Nikles, M. Piao, A.M. Lane, and D.E. Nikles, *Green Chem.*, **3**, 109 (2001).
64 M. Henneberry, J.A. Snively, G.J. Vasek, and R. Datta. **US Patent 6,797,684**.
65 H. Guo, W. Wang, Y. Sun, H. Li, F. Ai, L. Xie, and X. Wang, *J. Hazard. Mater.*, **174**, 59 (2010).
66 Y. Sun, L. Ji, W. Wang, X. Wang, and J. Wu, *J. Environ. Qual*., **38**, 1591 (2009).
67 R. Ferreira, N. Pedrosa, I.M. Marrucho, and L.P.N. Rebelo, *J. Chem. Eng*., **53**, 588 (2008).
68 J. Muse, and H.A. Colvin, **US Patent 0,287,179**.
69 R.J. Greff. **US Patent 6,051,607**.
70 C.M. Harvey. **US Patent 6,165,987**.
71 S.K. Gupta. **US Patent 0,110,415**.
72 Y-J. Wee, J-N. Kim, and H-W. Ryu, *Food Technol. Biotechnol.*, **44**, 163 (2006).
73 J. Drapeau, M. Verdier, D. Touraud, U. Kröckel, M. Geier, A. Rose, and W. Kunz, *Chem. Biodivers*., **6**, 934 (2009).
74 A. Dandia, A.K. Jain, and A.K. Laxkar, *Tetrahedron Lett*., **54**, 3929 (2013).
75 P.P. Ghosh, S. Paul, and A.R. Das, *Tetrahedron Lett*., **54**, 138 (2013).
76 L. Huang, Y. Zhu, H. Zheng, M. Du, and Y. Li, *Appl. Catal. A-Gen*., **349**, 204 (2008).
77 P. Kasinathan, J-W. Yoon, D.W. Hwang, U-H. Lee, J-S. Hwang, Y.K. Hwang, and J-S. Chang, *Appl. Catal. A-Gen.*, **451**, 236 (2013).
78 D.T. Vu, C.T. Lira, N.S. Asthana, A.K. Kolah, and D.J. Miller, *J. Chem. Eng*., **51**, 1220 (2006).
79 P. Delgado, M.T. Sanz, and S. Beltrán, *Fluid Phase Equilib*., **255**, 17 (2007).
80 M.E. Zakrzewska, M.S. Manic, E.A. Macedo, and V. Najdanovic-Visak, *Fluid Phase Equilib*., **320**, 38 (2012).
81 M.S. Manic, D. Villanueva, T. Fornari, E.A. Macedo, and V. Najdanovic-Visak, *J. Chem. Thermodyn*., **48**, 93 (2012).
82 K. Chylinski, and J. Gregorowicz, *J. Chem. Thermodyn*., **30**, 1131 (1998).
83 D.W. Cho, M.S. Shin, J. Shin, W. Bae, and H. Kim, *J. Chem. Eng. Data*, **56**, 3561 (2011).
84 A.B. Paninho, A.V.M. Nunes, A. Paiva, and V. Najdanovic-Visak, *Fluid Phase Equilib*., **360**, 129 (2013).
85 ZH. Zhao, and MH. Moghadasian, *Phytochem. Rev.*, **9**, 133 (2010).
86 E. Guerriero, A. Sorice, F. Capone, S. Costantini, P. Palladino, M. D'ischia, and G. Castello, *Molecules*, **16**, 6365 (2011).
87 B.L. Dhananjaya, A. Nataraju, C.D.R. Gowda, B.K. Sharath, and C.J.M. D'Souza, *Biochem.-Moscow*, **74**, 1315 (2009).
88 P.S.M. Prince, S. Rajakumar, and K. Dhanasekar, *Eur. J. Pharmacol*., **668**, 233-240 (2011).
89 D.D. Deb, G. Parimala, S.S. Devi, and T. Chakraborty, *Chem.-Biol. Interact*., **193**, 97 (2011).
90 V. Kumar, and G.A. Ravishankar, *Food Rev. Int*., **25**, 175 (2009).
91 J. Gmehling, J. Li, and M. Schiller, *Ind. Eng. Chem. Res*., **32**, 178 (1993).
92 H. Ashihara, and A. Crozier, *TRENDS Plant. Sci*., **6**, 407 (2001).

93 H.S. Park, H.J. Lee, M.H. Shin, K.W. Lee, H. Lee, Y.S. Kim, K.O. Kim, and K.H. Kim, *Food Chem*., **105**, 1011 (2007).
94 A. Perva-Uzunalic, M. Skerget, Z. Knez, B. Weinreich, F. Otto, and S. Gruner, *Food Chem*., **96**, 597 (2006).
95 E. Tedeschi, H. Suzuki, and M. Menegazzi, *Ann. N.Y. Acad. Sci.*, **973**, 435 (2002).
96 R. Cooper, D.J. Morre, and D.M Morre, *J. Altern. Complement. Med*., **11**, 521 (2005).
97 K. Zosel, *Chemie*, **90**, 748 (1978).
98 W-J. Kim, J-D. Kim, J. Kim, S-G. Oh, Y-W. Lee, *J. Food Eng.*, **89**, 303 (2008).
99 J. A. Mendiola, M. Herrero, A. Cifuentes, and E. Ibañez, *J. Chromatogr. A*, **1152**, 234 (2007).
100 B.K. Ishida, and G.E. Bartley, **Encyclopedia of human nutrition** 2nd ed., *Elsevier*, Oxford, 2005.
101 M. Zelkha, M. Ben-Yehuda, D. Hartal, Y. Raveh, and N. Garti, **US Patent 5,837,311**.
102 M. Kawaragi, T. Kuraishi, H. Shirasawa, N. Takada, N. Takada, Y. Katsumi, and S. Kojima, **US Patent 5,871,574**.
103 K. Bortlik, L. Mortezavi, and F. Saucy, **WO Patent 01/38443**.
104 H. Al-Wandawi, M. Abdul-Rahman, and K. Al-Shaikhly, *J. Agric. Food Chem*., **33**, 804 (1985).
105 W. Wu, M. Lu, and L. Yu, *Eur. Food. Res. Technol*., **232**, 463 (2011).
106 T. Nakano, M. Tosa, and M. Takeuchi, *J. Agric. Food Chem*., **43**, 1570 (1995).
107 M.G. Sajilata, R.S. Singhal, and M.Y. Kamat, *J. Food Eng*., **84**, 321 (2008).
108 T. Lu, F. Gaspar, R. Marriott, S. Mellor, C. Watkinson, B. Al-Duri, J. Seville, and R. Santos, *J. Supercrit. Fluids*, **41**, 68 (2007).
109 Z. Cohen, and Y. Heimer, **Industrial applications of single cell oils**, *AOCS Publishing*, 1992.
110 K. Dimas, D. Kokkinopoulos, C. Demetzos, B. Vaos, M. Marselos, M. Malamas, and T. Tzavaras, *Leukemia Res*., **23**, 217 (1999).
111 J. Ivanovic, D. Misic, I. Zizovic, and M. Ristic, *Food Control*, **25**, 110 (2012).
112 A.L. Dawidowicz, E. Rado, D. Wianowska, M. Mardarowicz, and J. Gawdzik, *Talanta*, **76**, 878 (2008).
113 A.L. Dawidowicz, E. Rado, and D. Wianowska, *J. Sep. Sci*., **32**, 3034 (2009).
114 M.R. García-Risco, G. Vicente, G. Reglero, and T. Fornari, *J. Supercrit. Fluids*, **55**, 949 (2011).

2

OBJETIVOS Y PLAN DE TRABAJO

2. OBJETIVOS Y PLAN DE TRABAJO

En la actualidad, café y té descafeinados se producen mediante extracción supercrítica así como utilizando disolventes líquidos. En cuanto a los disolventes líquidos, el uso de compuestos clorados ha ido disminuyendo hasta el punto de ser prácticamente reemplazados por agua o por disolventes orgánicos menos tóxicos y contaminantes, como el acetato de etilo. Estos disolventes más ecológicos presentan baja selectividad, especialmente el agua, produciéndose la extracción de compuestos implicados en las características sensoriales del alimento, así como compuestos con propiedades beneficiosas para la salud, disminuyendo la calidad del producto descafeinado. Particularmente, en el caso de la producción de té verde descafeinado es necesario minimizar la pérdida de catequinas, por ser estas las sustancias más importantes desde el punto de vista funcional de la bebida. En este sentido, incluso la extracción supercrítica, que constituye la más importante y reciente innovación en la extracción de alcaloides, conlleva una pérdida importante de estas sustancias durante el proceso de descafeinado.

Como ya se ha comentado, el lactato de etilo presenta interesantes propiedades para ser utilizado como disolvente, por su origen agroquímico, ser biodegradable y no tóxico. Estas características, junto a su capacidad para cubrir un amplio rango de polaridades, han provocado que actualmente se utilice como disolvente en diversos sectores industriales. No obstante, hay pocos estudios acerca del uso del lactato de etilo como disolvente de extracción de alimentos y ningún dato acerca de su uso como disolvente de extracción de alcaloides, y en particular, cafeína.

Las propiedades favorables del lactato de etilo y la demanda de disolventes ecológicos, pero eficientes y selectivos, para la extracción de ingredientes bioactivos de matrices vegetales, ha motivado el estudio que se presenta en esta memoria.

Los primeros objetivos se orientaron a la obtención de datos de equilibrio de fases y solubilidades, concretamente:

- Estudiar la solubilidad de sustancias alimentarias de interés funcional (incluyendo la cafeína) en lactato de etilo, con el fin de determinar su capacidad de extracción.

- Estudiar y definir el diagrama de fases líquido-vapor del sistema binario lactato de etilo + dióxido de carbono, para desarrollar procesos supercríticos de extracción y/o fraccionamiento que involucren el uso de dióxido de carbono y lactato de etilo.

Los resultados obtenidos permitieron presumir la eficacia del lactato de etilo en la extracción de cafeína de matrices alimentarias, sólo o combinado como cosolvente en la extracción supercrítica, y orientaron el trabajo de investigación de esta tesis, que lleva como título **"Lactato de etilo como nuevo disolvente verde para la extracción de cafeína de matrices vegetales"**.

Así, el **objetivo general** de esta tesis ha sido **evaluar el uso del lactato de etilo como disolvente de extracción de cafeína de matrices vegetales, en concreto, granos de café verde y hojas de té verde**.

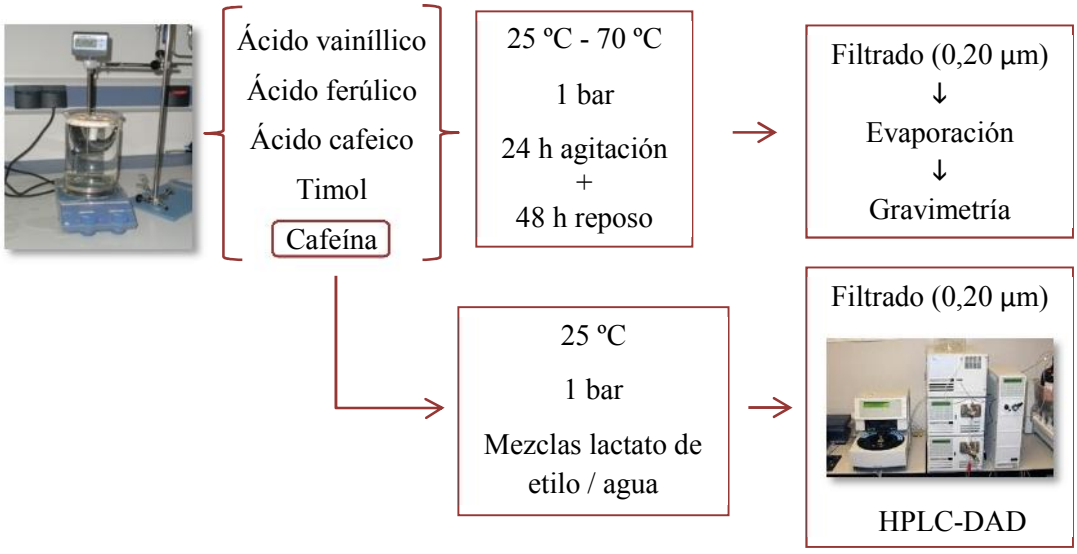
Los objetivos parciales definidos para desarrollar el estudio son los siguientes:

- Estudiar la selectividad y la eficacia del lactato de etilo en la extracción de cafeína de granos de café verde y hojas de té verde, estableciendo comparaciones con otros disolventes utilizados en la extracción comercial de este alcaloide.
- Estudiar la capacidad del lactato de etilo como cosolvente en la extracción supercrítica de cafeína de hojas de té verde, y compararla con otros cosolventes habitualmente utilizados.
- Desarrollar un proceso de fraccionamiento de un extracto de té verde utilizando dióxido de carbono supercrítico como gas antisolvente, con el objetivo de obtener un producto seco descafeinado con un alto contenido en catequinas, que pueda ser utilizado como ingrediente funcional.

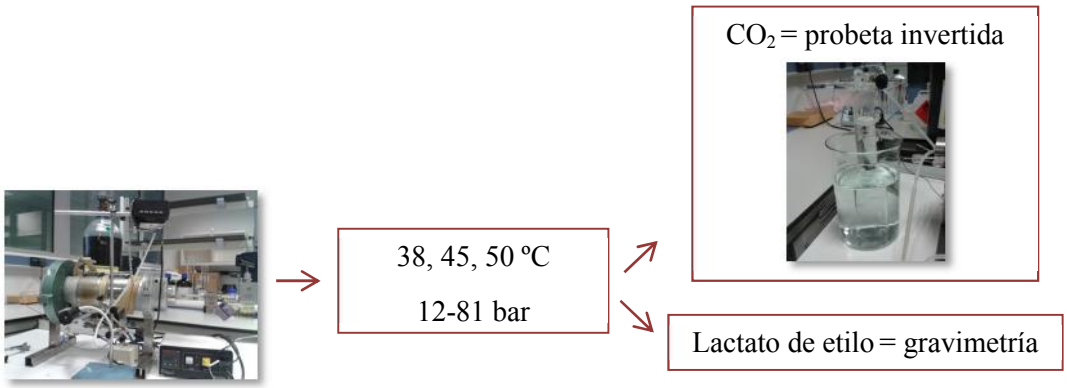
El plan de trabajo para alcanzar los objetivos de la tesis se resume en la siguiente figura.

Primera etapa: Medición de la solubilidad y del equilibrio de fases de sistemas que contienen lactato de etilo.

- Estudio de la solubilidad de sustancias alimentarias de interés funcional en lactato de etilo.

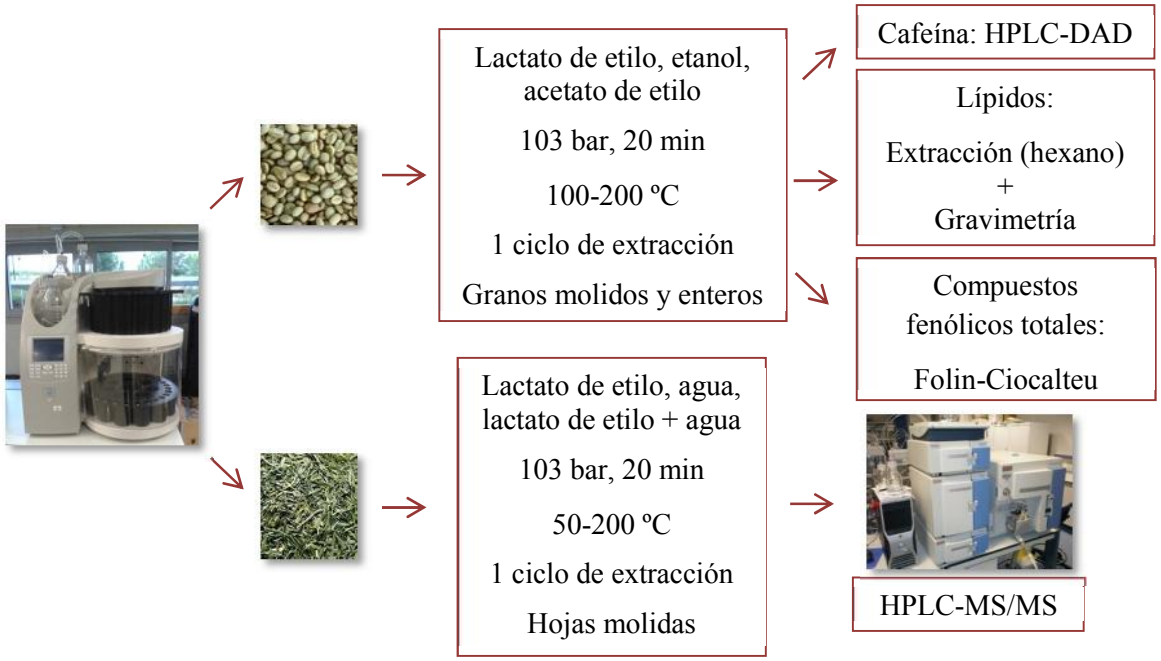


- Estudio del diagrama de fases líquido-vapor del sistema binario lactato de etilo + dióxido de carbono.

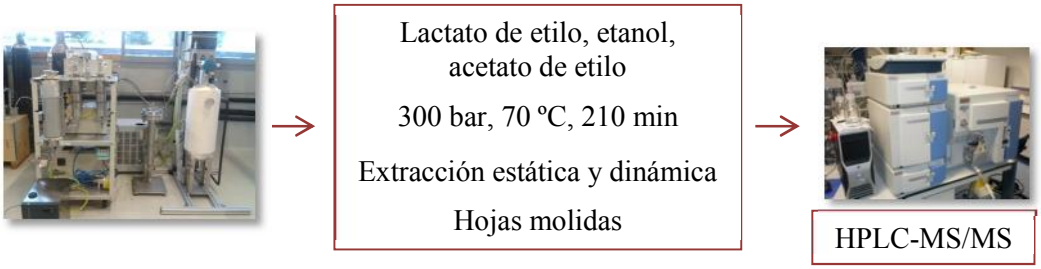


Segunda etapa: Estudio de la selectividad y la eficacia del lactato de etilo en la extracción de cafeína de granos de café verde y hojas de té verde.

- Estudio de la extracción de cafeína de granos de café verde y hojas de té verde utilizando lactato de etilo, etanol y acetato de etilo presurizado.



- Estudio de la extracción de cafeína de hojas de té verde utilizando dióxido de carbono supercrítico y lactato de etilo, etanol y acetato de etilo como modificadores.



Tercera etapa: Desarrollo de un proceso de fraccionamiento de un extracto de té verde utilizando dióxido de carbono supercrítico como gas antisolvente para la obtención de un extracto seco descafeinado con un alto contenido en catequinas.

Lactato de etilo, etanol
103 bar, 20 min, 100 ºC
1 ciclo de extracción
Hojas molidas

CO_2 como antisolvente
Lactato de etilo:
150-300 bar
Etanol:
100-300 bar
50 y 70 ºC

HPLC-DAD

3

RESULTADOS

3.1. Medición de la solubilidad y del equilibrio de fases de sistemas que contienen lactato de etilo

3.1.1. Solubilidad en lactato de etilo de varios compuestos alimentarios bioactivos

J. Chem. Thermodynamics 48 (2012) 93–100

Contents lists available at SciVerse ScienceDirect

J. Chem. Thermodynamics

journal homepage: www.elsevier.com/locate/jct

Solubility of high-value compounds in ethyl lactate: Measurements and modelling

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ARTICLE INFO

Article history:
Received 9 November 2011
Received in revised form 3 December 2011
Accepted 6 December 2011
Available online 13 December 2011

Keywords:
Solubility
Green solvents
High-value solutes
UNIQUAC
UNIFAC
Cubic-Plus-Association equation of state

ABSTRACT

(Solid + liquid) equilibria of binary mixtures containing high-value compounds and ethyl lactate were studied. Using the gravimetric method, the solubility of various solutes such as caffeine, vanillic acid, ferulic acid, caffeic acid and thymol in ethyl lactate was measured as a function of temperature over the range of (298.2 to 343.2) K, at atmospheric pressure. The differences in solubility of a given solute in water-saturated and dry ethyl lactate were observed. The deviation of these binary systems from ideal mixture behaviour was discussed. In order to understand the solubilization process, melting properties of pure solutes were determined by differential scanning calorimetry (DSC). The solubility data obtained were represented using UNIQUAC and UNIFAC-based models as well as with the Cubic-Plus-Association (CPA) equation of state. The results of the modeling indicate that these models are the appropriate tools for representing the solubility behaviour of various solutes in ethyl lactate.

1. Introduction

High-value compounds derived from natural sources are of industrial importance due to the increased perception of their health benefits associated with their antioxidant and antimicrobial activities. Some of the examples are derivatives of hydroxycinnamic acid, such as ferulic and caffeic acids, which are the most abundant phenolic acids found in seeds of many plants: cereals, coffee, fruits and vegetables. Studies have shown their potential in the prevention of chronic illnesses such as cardiovascular diseases and cancer [1]. Free ferulic and caffeic acids presented great antioxidant activities with high scavenging effect towards hydrogen peroxide, superoxide, hydroxyl radical and nitrogen dioxide free radicals [2]. This ability has an important role associated to the anti-cancer effect of these compounds. Kaul and Khanduja [3] reported that topical application containing caffeic and ferulic acids resulted in significant protection against anthracene-induced skin tumors while Guerriero *et al.* [4] showed anti-cancer activity of both acids on hepatocellular carcinoma. Ferulic acid significantly reduced the growth of oral cancer [5] as well as colon and rectal cancer [6].

Another example of phenolic compounds with high biological activity is vanillic acid which belongs to the hydroxybenzoic acid group. Recent bioactivity studies of hydroxy- and polyhydroxybenzoic acids were reviewed by Khadem and Marles [7]. Vanillic acid occurs in many plants and it is known for its anti-sickling and anthelmintic activities. It reduced hepatic fibrosis in chronic liver injury [8], inhibited snake venom 5′-nucleotidase [9] and showed the protective effects in isoproterenol induced cardiotoxic rats [10].

Thymol, a compound characteristic of essential oils, has been identified as an effective antibacterial with relatively low inhibitory concentrations in vitro and somewhat higher concentration in foods [11]. In the recent study [12], thymol demonstrated dose dependent cytotoxic effects on acute promyelotic leukemia cells after 24 h of exposure.

Furthermore, one of the natural products most widely consumed and studied in history is caffeine. Although research results are controversial, it is believed that low to moderate caffeine intake is generally associated with improvements in alertness, learning capacity, exercise performance, and possibly even in mood [13]. It is also used as an additive in pain medications.

Most of high-value compounds derived from natural sources are obtained by energetically intensive vacuum distillation including several additional steps associated with the use of abundant amounts of organic solvents. As an alternative, supercritical fluid technology has been applied to extract various high-value components from natural materials [14]. Nevertheless, despite good performances, large-scale supercritical applications are burdened with

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doi:10.1016/j.jct.2011.12.005

bulky equipment requirements. Consequently, the search for other new alternatives – those that would be less costly, more similar by structure to the classical solvents and yet ambient friendly – continues. In that respect, ethyl lactate is a green and economically viable alternative to traditional solvents: it is fully biodegradable, non-corrosive, non-carcinogenic and non-ozone depleting. Ethyl lactate is approved by the US Food and Drug Administration (FDA) as pharmaceutical and food additive and has been generally recognized as a safe (GRAS) solvent [15]. The molecular structure of ethyl lactate possesses a specific topology of hydrogen bonds present as well in other lactate alpha-hydroxyesters [16]. This allows the formation of intra- and intermolecular associations with ethyl lactate, as either proton donor or proton acceptor [17]. On the other hand, ethyl lactate is soluble in paraffin oils, which fact imposes the formation of some van der Waals interactions [18]. Thus, this ester offers diverse solvent properties that may cover a large number of solutes. Consequently, there are several attempts in the literature to use ethyl lactate as an extraction solvent. For example, Ishida and Chapman [19] reported the potential application of ethyl lactate to extract carotenoids from different sources, such as tomatoes, carrots and corn; Strati and Oreopoulou [20] studied the effect of different extraction parameters on the carotenoid recovery from tomato waste. A bioactive bicyclic diterpene, namely sclareol, was selectively extracted using ethyl lactate and recovered from the liquid solution by a CO_2 gas anti-solvent procedure [21]. Hernández *et al.* [22] studied the potential application of ethyl lactate to recover squalene from olive oil deodorizer distillates. Our group also reported the utilization of ethyl lactate for selective separation of α-tocopherol from triglycerides [23].

The solvent selection is one of the essential parameters to envisage any extraction process. Therefore, the knowledge of the solubility of a target component in different solvents is required. In this work, the solubility of caffeine, vanillic acid, ferulic acid, caffeic acid and thymol, in liquid ethyl lactate were measured over the temperature range (293.2 to 343.2) K. Although experimental data on solubility are essential to provide information about a system and help to understand its behaviour, correlations and prediction models are also required for the correct design of separation processes.

Binary systems containing ethyl lactate have been described by some models, such as UNIQUAC [22,23], UNIFAC activity coefficient models coupled with the Peng–Robinson equation of state (PR–EOS) [24] and the perturbed chain-statistical associating fluid theory (PC-SAFT) [24]. In this work, the obtained solubility data in ethyl lactate of caffeine, vanillic acid, ferulic acid, caffeic acid and thymol, were represented using the UNIQUAC model as well as the modified (Dortmund) UNIFAC method.

In addition, for the first time, we applied a simple Cubic Equation of State incorporating association, known as the CPA EoS for the description of the intermolecular physical interactions that include specific association in ethyl lactate containing systems. The CPA EoS was already applied successfully for binary mixtures (water + phenolic) compounds as reported by Mota *et al.* [25,26] and Queimada *et al.* [27].

2. Experimental

2.1. Materials

The following denote mass fraction purity of the materials. Caffeine (0.99), vanillic acid (0.97), ferulic acid (0.99), caffeic acid ($\geqslant$0.980), thymol ($\geqslant$0.995) and ethyl lactate (0.98) were supplied by Sigma–Aldrich (table 1). Their molecular structures are given in figure 1. All solutes were used without further purification. We studied solubility of solutes in: (a) water-saturated ethyl lactate as received and without any further treatment, and (b) dried ethyl lactate. In the case of latter, vacuum at room temperature was applied to ethyl lactate for several days in order to reduce its water content. Karl-Fischer coulometric titration (Metrohm 870 KF Titrino Plus coulometer) was employed to determine the water content before and after the vacuum procedure.

TABLE 1
Purity of the chemicals used in this work.

Compound	Supplier	CAS number	Sample purity, mass fraction
Ethyl lactate	Aldrich	687-47-8	0.98
Caffeine	Sigma–Aldrich	58-08-2	$\geqslant$ 0.99
Vanillic acid	Fluka	121-34-6	$\geqslant$ 0.97
Ferulic acid	Aldrich	537-98-4	0.99
Caffeic acid	Sigma	331-39-5	$\geqslant$ 0.98
Thymol	Sigma	89-83-8	$\geqslant$ 0.995

2.2. Experimental procedure

2.2.1. Differential scanning calorimetry

Differential scanning calorimetry (Netzsch, model DSC 200 F3 Maia) was used in order to obtain the melting point (T_m), enthalpy of fusion (ΔH_{fus}) and differences in heat capacities (ΔC_p) of caffeine, vanillic acid, ferulic acid, caffeic acid and thymol required for modeling the (solid + liquid) equilibrium. An aluminium crucible with (5 to 7) mg of sample was sealed hermetically and placed in the measuring cell of the calorimeter together with an empty crucible to be used as a reference. The sample was heated under a nitrogen stream over a large temperature range using a $3\ K \cdot min^{-1}$ heating rate. The measurements for each compound were repeated four times and average melting temperatures, enthalpies of fusion and differences in heat capacities were obtained.

2.2.2. (Solid + liquid) equilibria

For all the studied solutions, except the one with thymol, (solid + liquid) equilibrium measurements were carried out using the gravimetric method. Ethyl lactate and a solute (caffeine or vanilic acid or ferulic acid or caffeic acid) in excess were placed into a glass vessel with a stirrer. The vessels were put inside a water bath and a stirring plate was used to agitate the samples during 48 h under fixed temperature, controlled by a thermocouple (Julabo ED). The temperature was monitored by a calibrated mercury thermometer, having an accuracy of 0.1 K. After equilibrium had been reached, stirring was stopped and vessels were left stilled for more 48 h to allow a complete phase separation. Samples of clear saturated liquid solution (1 cm^3) were taken by a micropipette and placed into glass vials, while both the mass of the empty vial and the mass of the sample were registered using an AAA 250L balance with the precision of ±0.0001 g. The samples were then placed in a vacuum oven (Precision Scientific 5831) equipped with a vacuum pump (Edwards E2M1.5) for a couple of hours till constant mass of the dry samples were achieved. In order to evaporate all ethyl lactate from the samples, moderate temperature (338 K) and low pressure (1 Pa) were applied. The vials containing dry samples were weighted and the mole fraction solubilities were finally calculated.

In the case of (ethyl-lactate + thymol) solutions a visual dynamic method was used to measure the solubility of thymol. Solutions were prepared gravimetrically in the glass cell using an AAA 250L balance, with the precision of ±0.0001 g. After vigorous mixing, the cell (explained in details elsewhere [28,29]) was placed in the glass thermostat bath and the sample was heated very slowly (less than $0.5\ K \cdot h^{-1}$ near the equilibrium temperature) with continuous stirring. The temperature at which the last crystal disappeared was taken as that of (solid + liquid) equilibrium.

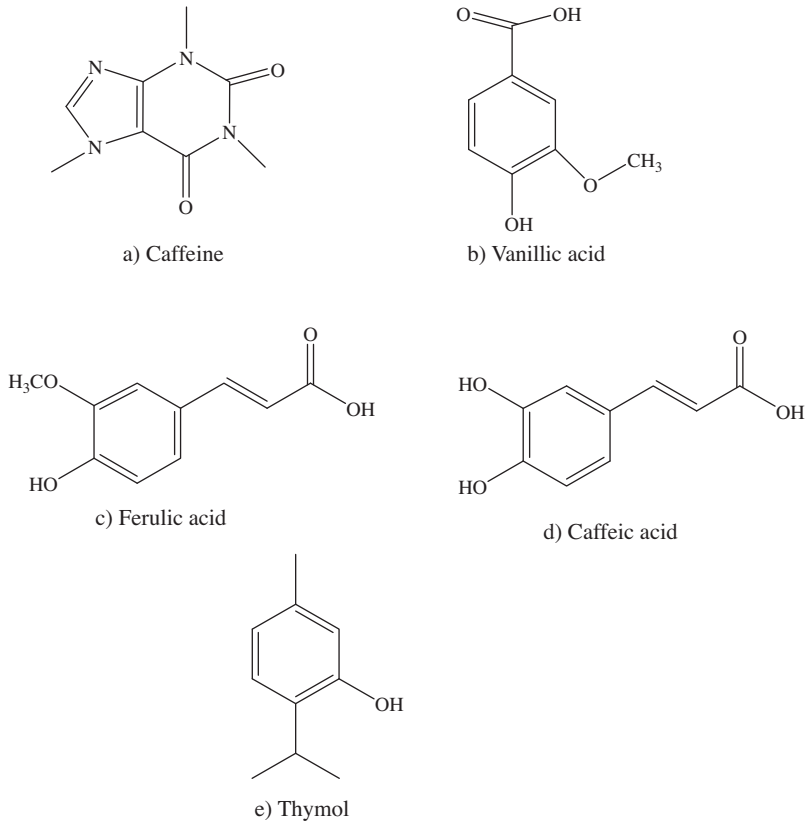

FIGURE 1. Chemical structure of caffeine (a), vanillic acid (b), ferulic acid (c), caffeic acid (d) and thymol (e).

For both methods, triplicates of each measurement were performed in order to obtain reliable solubility data. The average reproducibility in (solid + liquid) equilibrium temperature and compositions (mole fractions of solutes in ethyl lactate) was ±0.3 K and 0.0007, respectively.

2.3. Thermodynamic modelling

The solubility of a solute i in a liquid phase can be calculated by the following equation [30]:

$$\ln\left[\frac{f_i^{liq}(T,P)}{f_i^{sol}(T,P)}\right] = \sum_{tr}\frac{\Delta_{tr}H}{R}\left(\frac{1}{T}-\frac{1}{T_{tr}}\right) - \frac{\Delta C_p}{R}\left[\frac{T_m}{T} - \ln\left(\frac{T_m}{T}\right) - 1\right], \quad (1)$$

where $\Delta_{tr}H$, R, T and ΔC_p are the enthalpy of transition at the transition temperature (T_{tr}), the ideal gas constant, absolute temperature of (solid + liquid) equilibria, and difference of the liquid and solid molar heat capacities, respectively. $\Sigma\Delta_{tr}H$ integrates enthalpies of different solid–solid and fusion phase transitions of the solute.

In this work, the experimental solubility data were described by the UNIQUAC model [30] and by the modified UNIFAC (Dortmund) method – [31] as well as by the Cubic Plus Association equation of state (CPA EoS) [32,33].

The UNIQUAC equation [30] (an activity coefficient model) can be used to represent the solubility data and equation (1) then becomes:

$$x_i = \frac{1}{\gamma_i}\exp\left[-\sum_{tr}\frac{\Delta trH}{R}\left(\frac{1}{T}-\frac{1}{T_{tr}}\right) - \frac{\Delta C_p}{R}\left[\frac{T_m}{T} - \ln\left(\frac{T_m}{T}\right) - 1\right]\right], \quad (2)$$

where x_i and γ_i are the mole fraction of solute i in the liquid phase and the solute i activity coefficient.

The surface area and volume fraction used in UNIQUAC were based on the volume and area parameters which were calculated using the corresponding group contribution values [34,35]. The temperature-independent binary interaction parameters were obtained from the correlation of the SLE experimental data.

Equation (2) was also applied using the modified UNIFAC model [31] to calculate the solute activity coefficient in the liquid phase. The ACOH–COOH interaction parameters (both groups are present in the chemical structure of the phenolic acids studied) were estimated in this work using the SLE experimental data.

The Cubic Plus Association equation of state (CPA EoS) is a combination of the simple cubic equation of state (SCEoS) and the Wertheim association term. The SCEoS term presents the description of the physical interactions, while the Wertheim association term takes into account the specific association interactions between molecules. The CPA EoS can be expressed in terms of the compressibility factor, where the pure component energy parameter (a) is given by a Soave-type temperature dependence:

$$Z = z^{phys} + Z^{assoc} = \frac{1}{1-b\rho} - \frac{\alpha\rho}{RT(1+b\rho)} - \frac{1}{2}\left(1+\rho\frac{\partial \ln g}{\partial\rho}\right)\sum_i x_i \sum_{A_i}(1-X_{A_i}), \quad (3)$$

$$a(T) = a_0[1 + c_1(1-\sqrt{T_r})]^2, \quad (4)$$

where ρ and T_r are the molar density and reduced temperature.

The X_{Ai} is related to the association strength $\Delta^{A_iB_j}$ between sites A and B belonging to two different molecules (i,j). Since self- and cross-association are present in the systems studied, X_{Ai} is calculated through the following set of equations:

$$X_{A_i} = \frac{1}{1+\rho\Sigma_j x_j \Sigma_{B_j} X_{B_j}\Delta^{A_iB_j}}, \quad (5)$$

$$\Delta^{A_iB_j} = g(\rho)\left[\exp\left(\frac{\varepsilon^{A_iB_j}}{RT}\right) - 1\right] b_{ij}\beta^{A_iB_j}, \quad (6)$$

$$\Delta^{A_iB_j} = \sqrt{\Delta^{A_iB_i}\Delta^{A_jB_j}}, \quad (7)$$

$$g(\rho) = \frac{1}{1 - 1.9\eta}, \quad (8)$$

$$\eta = \frac{1}{4} b\rho. \quad (9)$$

Equation (6) is used for self-associating molecules where ε^{AiBi} and β^{AiBi} are the association energy and association volume, respectively. The Elliot combining rule (equation (7)) is used for cross-associating molecules.

The CPA EoS has been recently adopted for complex molecules in order to apply the explicit association energies and volumes for the different associating groups [25–27]. The CPA EoS has five pure component parameters (a_0, c_1, b, ε, β) for associating compounds, which are obtained by the simultaneous correlation of experimental liquid density and vapour pressure data, taking into account the number and type of associating groups. However, these experimental data were only available for ethyl lactate and thymol and they were collected from DIPPR Database [36]. Otherwise, the pure component parameters were calculated using the following equations proposed before for phenolics [25]:

$$a_0 = 0.2267 + 24.38\frac{T_c^2}{P_c}, \quad (10)$$

$$c_1 = -3.557 + (6.289 \times 10^{-3})T_c, \quad (11)$$

$$b = -2.328 \times 10^{-5} + 1.884 V_W, \quad (12)$$

where T_c, p_c and V_W are the critical temperature (in K), critical pressure (in Pa) and the van der Waals volume (in $m^3 \cdot mol^{-1}$), respectively.

The association energies and association volumes of ethyl lactate and thymol were as well determined using the pure component vapour pressure and liquid density data. The methodology described by Mota *et al.* [25] was used to obtain association energies and volumes for ferulic acid, vanillic acid and caffeic acid, since in these cases the vapour pressure and liquid density data were not available.

Finally, the values of the solubility of the solutes studied in ethyl lactate were obtained from the following equation:

$$x_i = \frac{\varphi_i^{liqo}}{\varphi_i^{liq}} \exp\left[-\sum_{tr}\frac{\Delta_{tr}H}{R}\left(\frac{1}{T} - \frac{1}{T_{tr}}\right) + \frac{\Delta C_p}{R}\left[\frac{T_m}{T} - \ln\left(\frac{T_m}{T}\right) - 1\right]\right] \quad (13)$$

in which the CPA EoS was used to calculate the fugacity coefficients. As mentioned before, the melting temperatures, enthalpies of fusion and differences in heat capacities were measured by DSC.

The experimental and modelling results were compared in terms of the absolute average deviations (AAD) of the solubility:

$$\mathrm{AAD}(\%) = \frac{1}{NP}\sum_i \frac{|x_i^{calc} - x_i^{exp}|}{x_i^{exp}} \times 100, \quad (14)$$

where x_i^{calc} and x_i^{exp} are the calculated and experimental mole fraction solubility respectively, and NP is the number of available solubility points.

3. Results and discussion

Measured enthalpies of fusion and melting temperatures along with differences in heat capacities for the studied solutes (caffeine, vanillic acid, ferulic acid, caffeic acid and thymol) are given in table 2.

TABLE 2
Average melting points (T_m), enthalpies of fusion (ΔH_{fus}) and differences in heat capacities (ΔC_p) of the compounds studied.*

Compound	T_m/K	ΔH_{fus}/(kJ · mol^{-1})	ΔC_p/(J · K^{-1} · mol^{-1})
Caffeine	405.8 ± 0.4[a]	2.6 ± 0.2[a]	
	505.4 ± 0.0	17.9 ± 0.1	12.0 ± 1.8
Vanillic acid	480.7 ± 0.2	29.1 ± 0.6	64.4 ± 2.5
Ferulic acid	444.9 ± 0.4	31.9 ± 0.9	73.7 ± 9.0
Caffeic acid	464.1[b]	39.85[b]	162.7[c]
Thymol	322.0 ± 0.1	17.4 ± 0.6	66.6 ± 4.7

* Maximal standard uncertainties u are $u(T_m) = 0.28$ K, $u(\Delta H_{fus}) = 0.6$, $u(\Delta C_p) = 6.4$.
[a] Solid–solid transition of caffeine.
[b] Calculated using a group contribution method as described elsewhere [25].
[c] Calculated using a group contribution method for the estimation of the heat capacities of liquids [38] and the power-law method to estimate heat capacities of organic solids [39].

A linear base line and a symmetric peak were observed for all the studied compounds, except for caffeine and caffeic acid. In the case of caffeine two phase transformations, solid–solid and solid–liquid, were detected upon heating while it was observed that caffeic acid decomposes before melting. Therefore, the melting point of caffeic acid adopted in this work was the one presented by Mota *et al.* [25] obtained by a third-order group-contribution method proposed by Marrero and Gani [37]. The difference in heat capacity of caffeic acid was acquired as a difference of the estimated liquid and solid heat capacities. The heat capacity of the li-

TABLE 3
Experimental values of the solubility of thymol, caffeine, vanillic acid, caffeic acid and ferulic acid in ethyl lactate containing 1.40 mass% of water and dried ethyl lactate containing less than 0.03 mass%.[a] x stands for solute mole fraction.

T/K	x	T/K	x
1.40 mass% water in ethyl lactate		<0.03 mass% water in ethyl lactate	
		Caffeine	
298.2	0.0192	296.2	0.0144
313.2	0.0305	303.1	0.0198
328.2	0.0418	312.7	0.0253
343.2	0.0508	323.0	0.0319
		333.3	0.0414
		Vanillic acid	
298.2	0.0270	296.2	0.0279
313.2	0.0355	303.1	0.0321
328.2	0.0482	312.7	0.0379
343.2	0.0584	323.0	0.0444
		333.3	0.0545
		Ferulic acid	
298.2	0.0803	296.2	0.0277
313.2	0.0939	303.1	0.0349
328.2	0.1061	312.7	0.0428
343.2	0.1177	323.0	0.0526
		333.3	0.0614
		Caffeic acid	
298.2	0.0129	296.2	0.0089
313.2	0.0165	303.1	0.0103
328.2	0.0203	312.7	0.0119
343.2	0.0230	323.0	0.0142
		333.3	0.0171
		Thymol	
301.4	0.6975	301.0	0.6978
304.3	0.7281	303.5	0.7207
307.5	0.7653	307.5	0.7638
307.8	0.7671	308.4	0.7784
308.3	0.7717	309.3	0.7928
316.5	0.8893	311.0	0.8085
318.6	0.9137	313.3	0.8421
		317.8	0.8985

[a] Standard uncertainties u are $u(T) = 0.15$ K, $u(x)$ for caffeine, vanillic acid, ferulic acid, caffeic acid equals to 0.0005, while for thymol equals to 0.0007.

quid as a function of temperature was estimated by the third-order group-contribution method given by Kolska *et al.* [38]. The temperature dependence of the group contribution was expressed as an empirical polynomial equation which applies the group contribution parameters determined by both a non-hierarchic and a hierarchic approach. As the non-hierarchic approach showed to be slightly superior, it was used to calculate the heat capacity of liquid caffeic acid. The heat capacity of solid caffeic acid was calculated using the power-law method which has a fixed temperature functionality but applies the two-group contribution method to obtain the compound-specific constant employed in the predictive equation [39].

The observed melting point of thymol was in a good agreement with the data reported in literature [40], showing a deviation of 0.7%. A substantially higher deviation was observed for its fusion enthalpy (20.9%). Similarly to what was observed by Dong *et al.* [41], caffeine showed two phase transitions, solid–solid and solid–liquid. In the case of the fusion of caffeine, our data deviated 0.7% and 9.9% for melting temperature and enthalpy of fusion, respectively. The properties of the solid–solid transition of caffeine also agreed reasonably with the literature data (1.8% and 22% deviations for melting point and fusion enthalpy, respectively). As for thymol and caffeine, the DSC thermograms of ferulic acid showed one endothermic peak and therefore corresponds to the one of two polymorphic forms reported by Sohn and Oh [42]. Measured melting temperature was smaller for 0.7% while the fusion enthalpy was higher for 22%.

Table 3 and figure 2 present the solubility data of caffeine, vanillic acid, ferulic acid, caffeic acid and thymol in ethyl lactate as a function of temperature. Since ethyl lactate is a hygroscopic compound, the solubility in both water-saturated (1.4 mass%) and dried (0.03 mass%) ethyl lactate was determined, thus permitting the understanding of the effect of water on solubility. To the best of our knowledge, there are no published data of the solubility of

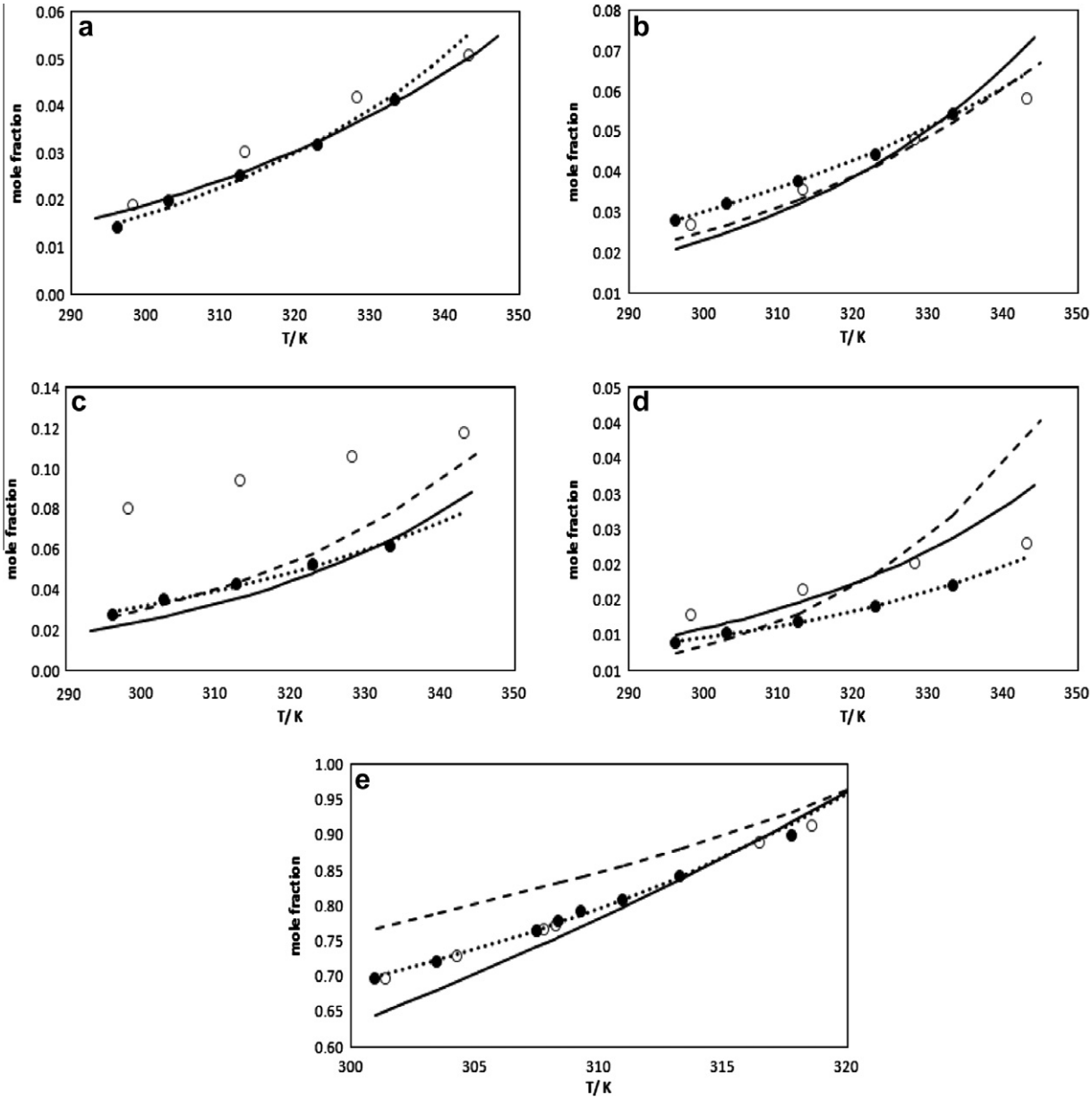

FIGURE 2. Plot of mole fraction against temperature to show the solubility of caffeine (a), vanillic acid (b), ferulic acid (c), caffeic acid (d) and thymol (e) in ethyl lactate: experimental results (empty circle stand for (solute + ethyl lactate) containing 1.40 mass% of water; filled circle stand for {solute + dried ethyl lactate system}). Lines present estimation by the UNIQUAC (round dot line), CPA (straight line) and UNIFAC (dashed line).

such given solutes in ethyl lactate with which to compare. The relative affinity of the studied solutes to ethyl lactate follows the order: thymol ≫ ferulic acid > vanillic acid > caffeine > caffeic acid. As expected, the solubility of all solutes studied in ethyl lactate were moderately enhanced by temperature rise. It was observed that thymol is extremely soluble in ethyl lactate, reaching mole fraction of 0.8985 at T = 317.8 K which can be explained by its relatively low melting point of 322.0 K and low enthalpy of fusion of 17.4 kJ · mol^{-1} (see table 2). Although the chemical structures of ferulic and caffeic acids (figure 1) are relatively similar, their solubility in ethyl lactate were quite unlike – 0.0614 and 0.0171 in mole fraction at T = 333.3 K for ferulic and caffeic acid, respectively.

The substitution of one hydroxyl group of caffeic acid by a methyl ether group enhanced the solubility significantly. The solubility of 0.0545 and 0.0614 in mole fraction at T = 333.3 K was observed for vanillic and ferulic acids, respectively. Thus, comparing these data it can be concluded that the presence of a longer acid alkyl chain increased the solubility only slightly.

It is interesting to note that the solubility of solutes was differently influenced by the presence of water in ethyl lactate solvent (figure 2). For example, the solubility of thymol was not changed by water while that of vanillic acid and caffeine was only slightly influenced. On the other hand, a significant increase of the solubility of ferulic and caffeic acids was observed when water was present in ethyl lactate. Taking into account a low solubility of ferulic and caffeic acids in water, this solubility enhancement suggests a co-solvent effect which may have implications in potential extraction processes.

According to equation (2), the calculation of the ideal solubility of a solute in a solvent at a given temperature is straightforward from the thermophysical property data (melting points, enthalpies of fusion and differences in heat capacities) of the compounds studied and presented in table 2. The ideal solubility corresponds to having an activity coefficient equal to one, meaning that the attractive forces between like-molecules (solvent–solvent and solute–solute) are the same as between unlike-molecules (solvent–solute). For the comparison of the deviation from ideal solution behaviour, it is convenient to present measured (real) solubility as a function of ideal solubility (figure 3). A straight dashed line corresponds to the ideal solution – activity coefficient $\chi_i = 1$. On the other hand, the area above this relates to the solubility higher than ideal, indicating a tendency toward ordering between the two unlike-molecule components ($\chi_i < 1$). Conversely, the area below the dashed line indicates a tendency toward phase separation or clustering in the solution, meaning that the attractive forces between like-molecules are superior to those of unlike-molecules ($\chi_i > 1$). For all the solutes studied except thymol, the activity coefficients were larger than unity, suggesting the presence of repulsive solute–solvent interactions. On the other hand, there are specific attractive forces between thymol and ethyl lactate, reflected in an activity coefficient lower than unity. Ferulic and vanillic acids showed a close to ideal behaviour at lower temperatures. As the temperature rises, solute–solvent interactions get weaker and are dominated by solute–solute and solvent–solvent cluster formations.

Calculated volume and area parameters of the UNIQUAC model (r_i and q_i) are included in table 4 along with the temperature-independent binary interaction parameters (a_{ij} and a_{ji}) obtained from fitting the experimental solubility data. The volume and area parameters are proportional to van der Waals volume (V_w) and van der Waals area (A_w) which are presented in table 5. As can be seen in figure 2, the UNIQUAC equation demonstrated an excellent description of the experimental results. The absolute average deviations comparing experimental and calculated solubility were 3.9% for caffeine, 0.98% for vanillic acid, 3.6% for ferulic acid, 0.97% for caffeic acid and 0.47% for thymol.

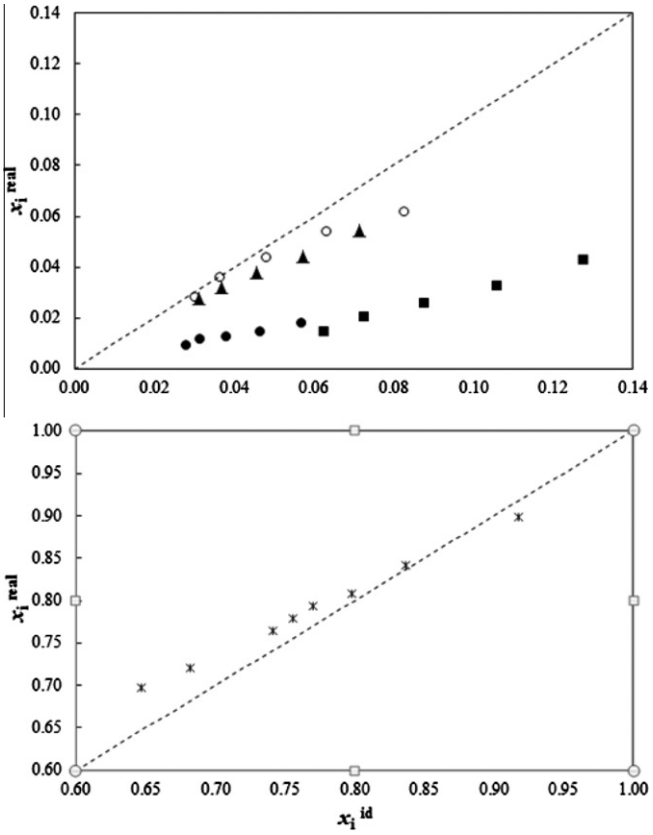

FIGURE 3. Plot of measured mole fraction against ideal mole fraction. The filled squares, filled triangles, empty circles, filled circles and asterisk stand for caffeine, vanillic acid, ferulic acid, caffeic acid and thymol, respectively. The straight dashed line corresponds to the ideal solution (activity coefficient $\gamma = 1$) calculated from equation (2), while areas above and below this line present region of $\gamma < 1$ and $\gamma > 1$, respectively.

TABLE 4
Interaction (a_{ij}, a_{ji}) and structural (r_i, q_i) parameters for the UNIQUAC model.

i	a_{ij}/K	a_{ji}/K	r_i	q_i
Ethyl lactate			4.441	3.928
Caffeine	409.11	−222.00	7.0534	5.6400
Vanillic acid	15.564	51.572	6.6638	5.6000
Ferulic acid	384.41	−207.61	5.8266	5.0040
Caffeic acid	380.93	−147.59	6.2624	5.1600
Thymol	459.35	−306.35	6.4931	4.8640

TABLE 5
Critical temperatures (T_c), critical pressures (p_c), van der Waals volumes (V_w) and van der Waals surface areas (A_w) used.

Compound	T_c/K	p_c/ MPa	$V_w \cdot 10^5$/ ($m^3 \cdot mol^{-1}$)	$A_w \cdot 10^{-6}$/ ($m^2 \cdot mol^{-1}$)
Ethyl lactate [36]	607.0	3.74	6.74	0.98
Caffeine [46]	855.6	4.15	10.11[36]	1.40[36]
Vanillic acid [45]	905.2	3.45	8.84[a]	1.25[a]
Ferulic acid [25]	854.6	3.64	10.70	1.41[a]
Caffeic acid [25]	876.2	5.11	9.50	1.29[a]
Thymol [36]	698.3	3.41	9.85	1.22

[a] Calculated using the group-contribution approach proposed by Bondy [34].

TABLE 6
Group composition adopted to represent the chemical structure of solutes and ethyl lactate for UNIFAC method.

	Ethyl lactate	Vanillic acid	Ferulic acid	Caffeic acid	Thymol
CH_3	2				2
CH_2	1				
CH					1
CH=CH			1	1	
AC		2	2	1	1
ACH		3	3	3	3
$ACCH_3$					1
ACOH		1	1	2	1
OH(s)	1				
CHCOO	1				
OCH_3		1	1		
COOH		1	1	1	

Table 6 shows the group composition of the substances studied in the case of applying the modified (Dortmund) UNIFAC model. The volume parameter (R_k) for the CHCOO group (present in ethyl lactate) was considered to be 1.2700, as is for the rest of groups comprising main group 11 (ester) given by Gmehling *et al.* [31]. The corresponding surface area parameter (Q_k) was calculated to be 0.9901, according to Bondi [43]. The rest of group R_k and Q_k parameters together with the temperature-dependent interaction parameters (a_{ij}, a_{ji}, b_{ij}, b_{ji}, c_{ij}, c_{ji}) were obtained from the literature [31].

In the case of thymol, the calculated values of solubility correspond to model predictions and give an absolute average deviation (AAD) between the experimental and calculated mole fractions of 6.9%. As mentioned before, the ACOH–COOH group interaction was estimated in this work, including non-zero b_{ij} and b_{ji} parameters, in order to represent the phenolic acid solubilities. The values obtained are given in table 7 along with a comparison with those reported in literature [31]. The AAD obtained between the experimental and calculated mole fractions were 11.4% for vanillic acid, 9.6% for ferulic acid and 24.7% for caffeic acid. Caffeine solubility could not be calculated due to the lack of parameter for cycl-CO group [44]. Figure 2 shows a comparison between the solubility calculations attained with the modified UNIFAC model and those obtained with the other models applied in this work.

The CPA pure component parameters for the solutes were calculated from available experimental values [25,36,45,46] according to equations (10)–(12). The van der Waals volume for vanillic acid was calculated using a group contribution approach proposed in literature [34]. All calculated and adopted data are presented in table 8.

The CPA EoS showed initially absolute average deviations (AAD) up to 72% when the pure component parameters were calculated according to equations (10)–(12). A small temperature-independent binary interaction parameter (k_{ij}) was thus necessary to decrease the AAD. The CPA modeling results thus obtained are presented in figure 2. The absolute average deviation for caffeine, vanillic acid, ferulic acid and caffeic acid are 6.05% ($k_{ij} = -0.043$), 13.71% ($k_{ij} = -0.213$), 14.97% ($k_{ij} = -0.022$) and 24.21% ($k_{ij} = -0.018$), respectively. The mixture of ethyl lactate and caffeic acid showed the highest AAD. The correlated k_{ij}'s are negative which means that the interactions between the molecules are stronger than expected by the CPA EoS. The ether group in vanillic acid was not taken into account for associative interactions which leads to the highest k_{ij} value. For the mixture of ethyl lactate and thymol, the CPA EoS gave a very small absolute average deviation (AAD = 3.17%) without adjusting the binary interaction parameter. This result leads to a conclusion that the CPA EoS is a good predictive tool for systems with self- and cross-association whenever binary interaction parameters cannot be obtained. It was also confirmed that the CPA EoS can still give satisfactory results if the pure component parameters of the solutes are obtained only from their molecular structure, whereas a small k_{ij} is the only parameter to be determined from experimental data.

TABLE 7
Modified UNIFAC interaction parameters between the ACOH and COOH groups: comparison between parameters regressed in this work and those reported in the literature.

i	*j*	a_{ij}	b_{ij}	c_{ij}	Ref.
ACOH	COOH	401.88	0.0	0.0	[31]
		415.72	−1.97	0.0	This work
COOH	ACOH	281.08	0.0	0.0	[31]
		120.50	−2.37	0.0	This work

4. Conclusions

In this work, the solubility of caffeine, vanillic acid, ferulic acid, caffeic acid and thymol in both dry and water saturated ethyl lactate was measured as a function of temperature, at atmospheric pressure. All values of the solubility were found to increase with temperature. Thermophysical properties of the studied solutes, namely, enthalpies of fusion and melting temperatures along with differences in heat capacities were obtained by DSC. From the thermophysical and solubility data, activity coefficients were calculated. It was found that for all the solutes studied except thymol, the activity coefficients were larger than unity, suggesting the presence of repulsive solute-solvent interactions. On the other hand, there are specific attraction forces between thymol and ethyl lactate, reflecting in activity coefficients lower than unity.

The solubility data obtained were represented using UNIQUAC and UNIFAC as well as using the Cubic-Plus-Association (CPA) equation of state. The UNIQUAC model provided an excellent description of the solubility data, with the absolute average deviations (AAD) of 3.9% for caffeine, 0.98% for vanillic acid, 3.6% for ferulic acid, 0.97% for caffeic acid and 0.47% for thymol. The UNIFAC-based model showed reasonable predictive capabilities for the mixtures studied. Good agreement between the experimental and calculated mole fractions were obtained for vanillic acid (AAD of 11.4%), ferulic acid (AAD of 9.6%), and thymol (AAD of

TABLE 8
Pure component parameters used in the CPA EoS.

Compound	$a_0/(\mathrm{Pa \cdot m^6 \cdot mol^{-2}})$	c_1	$b \cdot 10^4/(\mathrm{m^3 \cdot mol^{-1}})$	OH		COOH		% AAD	
				$\varepsilon \cdot 10^{-4}/(\mathrm{J \cdot mol^{-1}})$	$\beta \cdot 10^2$	$\varepsilon \cdot 10^{-4}/(\mathrm{J \cdot mol^{-1}})$	$\beta \cdot 10^3$	*p*	ρ
Ethyl lactate	1.994	1.030	1.030	1.875	4.046			0.513	0.062
Caffeine	4.532	1.824	1.672						
Vanillic acid	6.017	2.136	1.432	1.837	1.185	3.201	0.010		
Ferulic acid	5.118	1.818	1.783	1.871	1.345	2.756	3.698		
Caffeic acid	3.890	1.953	1.557	1.134	6.255	2.756	3.698		
Thymol	3.113	1.140	1.418	2.242	3.796			0.396	0.019

6.9%) while somewhat inferior agreement was observed for caffeic acid (AAD of 24.7%).

The CPA EoS represented very well the (solid + liquid) equilibrium data of the solutes studied, namely caffeine, vanillic acid, ferulic acid, caffeic acid and thymol in ethyl lactate, but only when a small binary interaction parameter was regressed from the experimental solubility data. The CPA modelling results for such complex molecules are surprisingly good, given the higher predictive character of the CPA EoS when compared with the activity coefficient models. It also clearly shows the importance of including associative effects in the model.

Acknowledgments

This work has been supported by *Fundação para a Ciência e a Tecnologia* through Grant No. PEst-C/EQB/LA0006/2011. The authors gratefully acknowledge the financial support from the Conselho de Reitores das Universidades Portuguesas (CRUP) – Integrated project Portugal – Spain, No. E-95/10 and from the Ministerio de Ciencia e Innovación of Spain (integrated project Spain-Portugal, PT2009-0010). M.S. Manic is thankful to Fundação para a Ciência e Tecnologia – Portugal for a doctoral fellowship (SFRH/BD/45323/2008). D. Villanueva thanks for the JAE-pre fellowship given by Consejo Superior de Investigaciones Científicas (CSIC) of Spain.

References

[1] Z. Zhao, M.H. Moghadasian, Phytochem. Rev. 9 (2010) 133–145.
[2] S. Ou, Y. Li, K. Gao, Acta Nutr. Sin. 21 (1999) 191–194.
[3] A. Kaul, K.L. Khanduja, Nutr. Cancer 32 (1998) 81–85.
[4] E. Guerriero, A. Sorice, F. Capone, S. Costantini, P. Palladino, M. D'ischia, G. Castello, Molecules 16 (2011) 6365–6377.
[5] H. Mori, K. Kawabata, N. Yoshimi, T. Tanaka, T. Murakami, T. Okada, H. Murai, Anticancer Res. 19 (1999) 3775–3778.
[6] K. Kawaba, T. Yamamoto, A. Hara, M. Shimizu, Y. Yamada, K. Matsunaga, T. Tanaka, H. Mori, Cancer Lett. 157 (2000) 15–21.
[7] S. Khadem, R.J. Marles, Molecules 15 (2010) 7985–8005.
[8] A. Itoh, K. Isoda, M. Kondoh, M. Kawase, M. Kobayashi, M. Tamesada, K. Yagi, Biol. Pharm. Bull. 32 (2009) 1215–1219.
[9] B.L. Dhananjaya, A. Nataraju, C.D.R. Gowda, B.K. Sharath, C.J.M. D'Souza, Biochem. Moscow 74 (2009) 1315–1319.
[10] P.S.M. Prince, S. Rajakumar, K. Dhanasekar, Eur. J. Pharmacol. 668 (2011) 233–240.
[11] P.C. Braga, M. Dal Sasso, M. Culici, T. Bianchi, L. Bordoni, L. Marabini, Pharmacology 77 (2006) 130–136.
[12] D.D. Deb, G. Parimala, S. Saravana Devi, T. Chakraborty, Chem. Biol. Interact. 193 (2011) 97–106.
[13] V. Kumar, G.A. Ravishankar, Food Rev. Int. 25 (2009) 175–197.
[14] M. Herrero, J.A. Mendiola, A. Cifuentes, E. Ibánez, J. Chromatogr. A 1217 (2010) 2495–2511.
[15] C.S.M. Pereira, V.M.T.M. Silva, A.E. Rodrigues, Green Chem. 13 (2011) 2658–2671.
[16] S. Aparicio, R. Alcalde, J. Phys. Chem. B 113 (2009) 14257–14269.
[17] S. Aparicio, S. Halajian, R. Alcalde, B. Garcıa, J.M. Leal, Chem. Phys. Lett. 454 (2008) 49–55.
[18] J. Drapeau, M. Verdier, D. Touraud, U. Krockel, M. Geier, A. Rose, W. Kunz, Chem. Biodivers. 6 (2009) 934–947.
[19] B.K. Ishida, M.H.J. Chapman, Agric. Food Chem. 57 (2009) 1051–1059.
[20] I.F. Strati, V. Oreopoulou, Int. J. Food Sci. Technol. 46 (2011) 23–29.
[21] X.C. Tombokan, R.M. Aguda, D.A. Danehower, P.K. Kilpatrick, R.G. Carbonell, J. Supercrit. Fluids 45 (2008) 146–155.
[22] E.J. Hernández, P. Luna, R.P. Stateva, V. Najdanovic-Visak, G. Reglero, T. Fornari, J. Chem. Eng. Data 56 (2011) 2148–2152.
[23] G. Vicente, A. Paiva, T. Fornari, V. Najdanovic-Visak, Chem. Eng. J. 172 (2011) 879–884.
[24] R. Ferreira, N. Pedrosa, I.M. Marrucho, L.P.N. Rebelo, J. Chem. Eng. Data 53 (2008) 588–590.
[25] F.L. Mota, A.J. Queimada, S.P. Pinho, E.A. Macedo, Ind. Eng. Chem. Res. 47 (2008) 5182–5189.
[26] F.L. Mota, A.J. Queimada, S.P. Pinho, E.A. Macedo, Fluid Phase Equilib. 303 (2011) 62–70.
[27] A.J. Queimada, F.L. Mota, S.P. Pinho, E.A. Macedo, J. Phys. Chem. B 113 (2009) 3469–3476.
[28] C.A.S. Trindade, Z.P. Visak, R. Bogel-Łukasik, E. Bogel-Łukasik, M. Nunes da Ponte, Ind. Eng. Chem. Res. 49 (2010) 4850–4857.
[29] M.S. Manic, V. Najdanovic-Visak, J. Chem. Thermodyn. 44 (2012) 102–106.
[30] J.M. Prausnitz, R.N. Lichtenthaler, E.G. Azevedo, Molecular Thermodynamics of Fluid-Phase Equilibria, Prentice-Hall PTR, Upper Saddle River, NJ, 1999.
[31] J. Gmehling, J. Li, M. Schiller, Ind. Eng. Chem. Res. 32 (1993) 178–193.
[32] G.M. Kontogeorgis, M.L. Michelsen, G.K. Folas, S. Derawi, N. Von Solms, E.H. Stenby, Ind. Eng. Chem. Res. 45 (2006) 4855–4868.
[33] G.M. Kontogeorgis, M.L. Michelsen, G.K. Folas, S. Derawi, N. Von Solms, E.H. Stenby, Ind. Eng. Chem. Res. 45 (2006) 4869–4878.
[34] A. Bondy, J. Phys. Chem. 68 (1964) 441–451.
[35] G.M. Kontogeorgis, G.K. Folas, Thermodynamic Models for Industrial Application: From Classical and Advanced Mixing Rules to Association Theories, John Wiley & Sons, Ltd., 2010.
[36] R.L. Rowley, W.V. Wilding, J.L. Oscarson, Y. Yang, N.F. Giles, DIPPR® Data Compilation of Pure Chemical Properties, Design Institute for Physical Properties, AIChE, New York, 2010.
[37] J. Marrero, R. Gani, Fluid Phase Equilib. 183 (2001) 183–208.
[38] Z. Kolska, J. Kukal, M. Zabransky, V. Ruzicka, Ind. Eng. Chem. Res. 47 (2008) 2075–2085.
[39] B.T. Goodman, W.V. Wilding, J.L. Oscarson, R.L. Rowley, J. Chem. Eng. Data 49 (2004) 24–31.
[40] J.S. Chickos, C.M. Braton, D.G. Hesse, J.F. Liebman, J. Org. Chem. 56 (1991) 927–938.
[41] X. Dong, Q. Li, Z.C. Tan, Z.-H. Zhang, Y. Liu, J. Chem. Thermodyn. 39 (2007) 108–114.
[42] Y.T. Sohn, J.H. Oh, Arch. Pharm. Res. 26 (2003) 1002–1008.
[43] A. Bondi, Physical Properties of Molecular Crystans, Liquids and Glasses, Wiley, New York, 1968.
[44] S. Nebig, J. Gmehling, Fluid Phase Equilib. 302 (2011) 220–225.
[45] A. Stassi, R. Bettini, A. Gazzaniga, F. Giordano, A. Schiraldi, J. Chem. Eng. Data 45 (2000) 161–165.
[46] G. Xu, A.M. Scurto, M. Castier, J.F. Brennecke, M.A. Stadtherr, Ind. Eng. Chem. Res. 39 (2000) 1624–1636.

JCT -11-516

3.1.2. Equilibrio de fases del sistema binario CO_2 – lactato de etilo

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Solubility of CO_2 in Ethyl Lactate and Modeling of the Phase Behavior of the CO_2 + Ethyl Lactate Mixture

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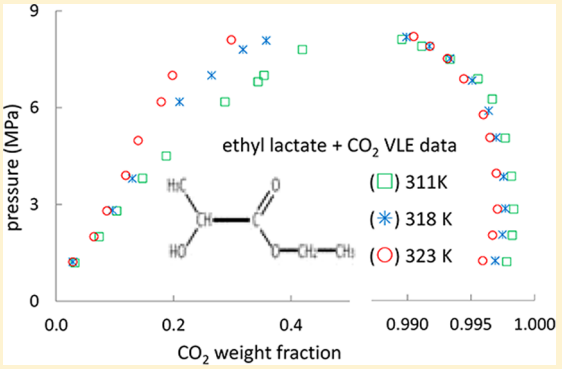
ABSTRACT: Ethyl lactate (ethyl 2-hydroxypropanoate) is emerging as an ecofriendly solvent with wide applications in the food, pharmaceutical, and fine chemical industries. Furthermore, its potential use as a solvent in chemical reactions which could be performed in supercritical media and as an effective cosolvent in supercritical extraction processes and/or in antisolvent precipitation processes has also been studied. In view of this, knowledge of the phase behavior of ethyl lactate + CO_2 binary is vital for the modeling and design of such processes. In this work, data on the solubility of CO_2 in the ethyl lactate rich liquid phase at T = (311, 318, and 323) K and pressures ranging from (1 to 8.1) MPa is reported for the first time. The compositions of the liquid phase were measured using a variable volume view equilibrium cell. The new data measured together with previously reported data on the solubility of ethyl lactate in CO_2 were employed to explore the capabilities of two different thermodynamic models, namely, the cubic Soave−Redlich−Kwong equation of state (SRK-EoS) and the group contribution EoS (GC-EoS), to represent the phase equilibria of the binary ethyl lactate + CO_2 mixture.



INTRODUCTION

Ethyl lactate (ethyl 2-hydroxypropanoate) has very interesting physicochemical properties which allow its application as an extractive solvent, cleaning agent, excipient, dispersing agent, and solvent reaction medium. Further, it has good ecological properties since it is produced by fermentation from corn feedstock: it is fully biodegradable, noncorrosive, noncarcinogenic, and nonozone depleting. Thus, it was accepted as GRAS (generally recognized as safe) and permitted by the U.S. Food and Drug Administration (FDA) as a pharmaceutical and food additive.

Using the instruments of modern computer-aided molecular design,[1] ethyl lactate was identified as a potential new environmentally friendly solvent. For example, it is a desirable coating for wood, polystyrene, and metals and is a cleaning agent for the polyurethane industry and for metal surfaces. Further, ethyl lactate can efficiently remove greases, oils, adhesives, fuels, and copper from contaminated soils[2] and is especially useful in pharmacy as a dispersing agent of biologically active compounds.[3,4]

Several new potential applications of ethyl lactate as an alternative green solvent in the food industry have been lately reported in the literature. The extraction of carotenoids from tomatoes, carrots, and corn,[5,6] the recovery of sclareol from salvia,[7] and γ-linolenic acid from Spirulina microalgae[8] are just some of the examples. Further, ethyl lactate applications connected with the edible oil industry were studied by us, such as the isolation of squalene from olive oil deodorizer distillates[9] and selective separation of α-tocopherol from triglycerides.[10] Moreover, the solubility of several bioactive compounds (e.g., caffeine, vanillic acid, ferulic acid, caffeic acid, and thymol) in liquid ethyl lactate at atmospheric pressure and in the temperature range of T = (298.2 to 343.2) K have also been reported recently.[11]

Indeed, there is a clear-cut need for obtaining detailed knowledge on the thermodynamics of the phase equilibria of systems comprising ethyl lactate as such data is a vital element in the reliable design and optimization of separation processes in which it could be applied. For example, ethyl lactate can be used as a solvent in chemical reactions in the food and pharmaceutical industry,[13] which are performed in supercritical media, and as a potential useful cosolvent in supercritical extraction processes and/or in antisolvent precipitation processes.[7]

In this work we focus on the binary system ethyl lactate + carbon dioxide (CO_2) and report for the first time data on the solubility of CO_2 in the ethyl lactate rich liquid phase at T = (311, 318, and 323) K and pressures ranging from (1 to 8.1) MPa. The solubility of ethyl lactate in CO_2 has been measured previously at the same temperatures and in the same range of

Received: August 2, 2012
Accepted: December 20, 2012
Published: January 7, 2013

pressures,[12] but no data on the liquid phase equilibrium compositions were presented.

Additionally, the liquid phase compositions measured in this work and the solubility data reported by Chylinski and Gregorowicz[12] were represented using two well-known thermodynamic models: the group contribution equation of state (GC-EoS) and the Soave−Redlich−Kwong cubic EoS (SRK-EoS). Model equations and a detailed explanation of their fundamentals are given elsewhere.[14,15]

EXPERIMENTAL SECTION

Materials. Ethyl lactate (≥ 99 % purity) was obtained from Sigma-Aldrich (St. Louis, MO, USA). High-purity CO_2 (more than 99.9 % in volume purity, SFC grade) supplied by AIR LIQUIDE was used as received.

Equipment and Experimental Procedure. A high-pressure equilibrium cell was employed to perform liquid-phase composition measurements. A full description of the equipment is given elsewhere.[16]

Ethyl lactate and CO_2 were introduced into the equilibrium cell after purging with CO_2. The temperature was stabilized, and the pressure was attained using the manual pressure generator. Stirring was turned on and maintained for 1.5 h. Then, the mixture was let to repose for 3 h so that complete phase segregation occurred. Stirring and repose times were selected according to a previous work.[16]

Samples from the equilibrium liquid phase were decompressed to atmospheric pressure via a thermostatized capillary line and a micrometering valve, while the manual pressure generator was used to keep pressure constant during sampling (± 0.05 MPa). A cool trap placed after the valve was employed to separate ethyl lactate from CO_2. Ethyl lactate was collected in vials, which were weighed on a precision analytical balance (0.0001 g accuracy) to determinate the mass of ethyl lactate recovered. CO_2 was conducted to a 100 mL graduated tube filled with and placed into a saturated water solution of Na_2SO_4 to prevent CO_2 dissolution. The amount of CO_2 recovered from the sample was measured as the displaced solution volume (± 1 mL of precision) and corrected by the effect of water vapor pressure.

The uncertainty in the CO_2 mass fractions (w_{CO_2}) measured was calculated as the average standard deviation (ASD) between the values obtained in duplicate samples:

$$\mathrm{ASD} = (1/2)\sqrt{(1/N_{\mathrm{exp}})\sum (w_{CO_2}^{I} - w_{CO_2}^{II})^2} \quad (1)$$

where I and II represent the duplicate samples and N_{exp} is the total number of experimental data points measured. The calculated ASD value of CO_2 mass fractions in the liquid phase was 0.0008.

Thermodynamic Modeling Framework. At the temperatures and pressures studied, the binary system ethyl lactate + CO_2 exhibits vapor−liquid equilibria.

For vapor−liquid equilibrium the general equilibrium relation is:

$$f_i^{L} = f_i^{V} \quad (2)$$

where f_i^{L} and f_i^{V} are the fugacities of component i in the liquid and vapor phases, respectively.

Provided the so-called "$\varphi-\varphi$" approach is used to model the fugacities of the components in the equilibrium liquid and vapor phases the following holds:

$$f_i^{L} = x_i \varphi_i^{L} p$$
$$f_i^{V} = y_i \varphi_i^{V} p \quad (3)$$

where p is the pressure, x_i and y_i are mole fractions, and φ_i^{L} and φ_i^{V} are fugacity coefficients of the i-th component in the liquid and vapor phases, respectively.

To calculate the fugacity coefficient of the i-th component in the liquid and vapor phase, respectively, we apply two thermodynamic models, representatives of the EoS family and valid over the entire density range studied: the first one is predictive and the other is correlative by nature, namely, the GC-EoS[14] model and the SRK cubic EoS.[15]

The GC-EoS Model. The GC-EoS has two contributions to the residual Helmholtz energy of the system: a repulsive hard sphere Carnahan−Starling type term and an attractive term, which combines the group contribution approach with the local-composition mixing rules. The model parameters are the following: the critical hard sphere diameter (d_c) which is characteristic of each substance, five pure-group parameters (T^*, q, g^*, g', and g'') and four binary interaction parameters (k_{ij}^*, k_{ij}', α_{ij}, and α_{ji}). The reference temperature (T^*) and group surface area (q) are not adjustable parameters (q calculated according to Bondi[17]), while the pure group parameters (g^*, g', and g'') are usually regressed using adequate vapor pressure data. A complete explanation of the model is given by Skjold-Jørgensen.[14]

The SRK-EoS Model. The SRK-EoS[15] was applied with the two-binary interaction-parameter-per-pair (2PWDW) version of the van der Waals one fluid mixing rule. The expressions for the cross-energy and for the cross-co-volume parameters are, respectively:

$$a_{ij} = (a_{ii}a_{jj})^{0.5}(1 - k_{ij}) \quad (4)$$

$$b_{ij} = \left(\frac{b_{ii} + b_{jj}}{2}\right)(1 - l_{ij}) \quad (5)$$

In the above, k_{ij} and l_{ij} are the energy and size binary interaction parameters, respectively.

RESULTS AND DISCUSSION

Liquid-Phase Composition Measurement of the Ethyl Lactate + CO_2 System. The results of the liquid-phase composition measurements obtained in this work are reported in Table 1. Ethyl lactate weight fractions given in Table 1 correspond to mean values of the duplicate experiments. As expected, ethyl lactate weight fractions in the liquid phase increase as temperature increases and pressure decreases.

Figure 1 represents the isothermal liquid-phase compositions measured in this work, together with the vapor-phase compositions (solubility) data reported by Chylinski and Gregorowicz[12] in terms of CO_2 weight fractions.

Phase Equilibria Modeling Using the GCA-EoS. Figure 2 shows the chemical structure of ethyl lactate. According to the current GC-EoS parameter table,[18] the group composition of ethyl lactate molecule can be described by means of two CH_3, one CH_2COO, and one CHOH group (set 1). Nevertheless, considering that ethyl lactate is the ester of lactic acid, a more appropriate representation should include the definition of a new alcohol-ester functional group (CHOH-COO) (see Figure 2); thus the ethyl lactate molecule will be

dx.doi.org/10.1021/je301039e | J. Chem. Eng. Data 2013, 58, 301−306

Table 1. Liquid-Phase Composition (Mass Fraction) of the Binary System Ethyl Lactate (1) + CO_2 (2) at T = (311, 318, and 323) K[a]

T/K	w_2	p/MPa
311	0.032	1.2
311	0.074	2.0
311	0.103	2.8
311	0.147	3.8
311	0.188	4.5
311	0.288	6.2
311	0.344	6.8
311	0.354	7.0
311	0.419	7.8
318	0.029	1.2
318	0.096	2.8
318	0.130	3.8
318	0.210	6.2
318	0.265	7.0
318	0.318	7.8
318	0.358	8.1
323	0.029	1.2
323	0.065	2.0
323	0.087	2.8
323	0.119	3.9
323	0.140	4.9
323	0.179	6.2
323	0.198	7.0
323	0.299	8.1

[a]Standard uncertainties u are $u(T)$ = 0.1 K, $u(p)$ = 0.05 MPa, and $u(w_2)$ = 0.0008.

represented by two CH_3 groups, one CH_2 group, and the new CHOHCOO group (set 2).

Figure 3 shows the prediction of ethyl lactate vapor pressures using set 1 of groups and the corresponding parameters reported in the literature.[19] As can be seen, large deviations exist between the experimental[19] and calculated vapor pressures.

The absolute average deviation:

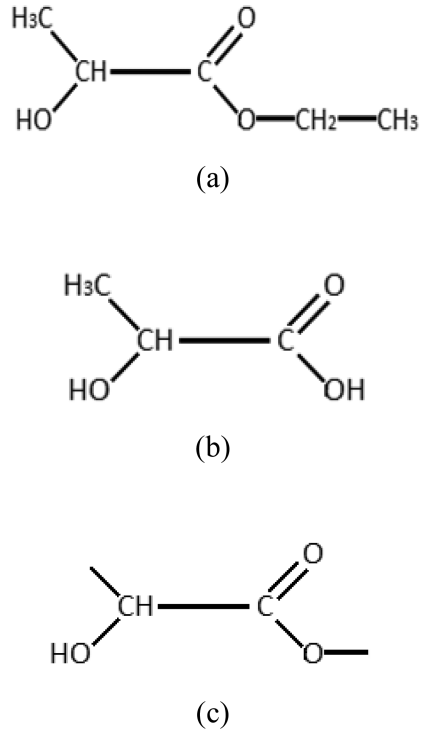


Figure 2. Chemical structure of (a) ethyl lactate, (b) lactic acid, and (c) the alcohol−ester group.

$$\mathrm{AAD} = \frac{1}{N}\sum\left(\frac{p_{\mathrm{exp}}^{\mathrm{vap}} - p_{\mathrm{cal}}^{\mathrm{vap}}}{p_{\mathrm{exp}}^{\mathrm{vap}}}\right) \quad (6)$$

is 96.7 % in the temperature range T = (320 to 370) K, even though the hard sphere diameter d_c of ethyl lactate was optimized (d_c = 5.2168 $cm^3 \cdot mol^{-1}$) to fit the vapor pressure experimental data.[19]

The new ester−alcohol group (CHOHCOO) was then employed to improve the GC-EoS representation of the ethyl lactate vapor pressures. The CHOHCOO pure group energy parameters and binary paraffin-CHOHCOO interaction parameters were simultaneously adjusted to the experimental

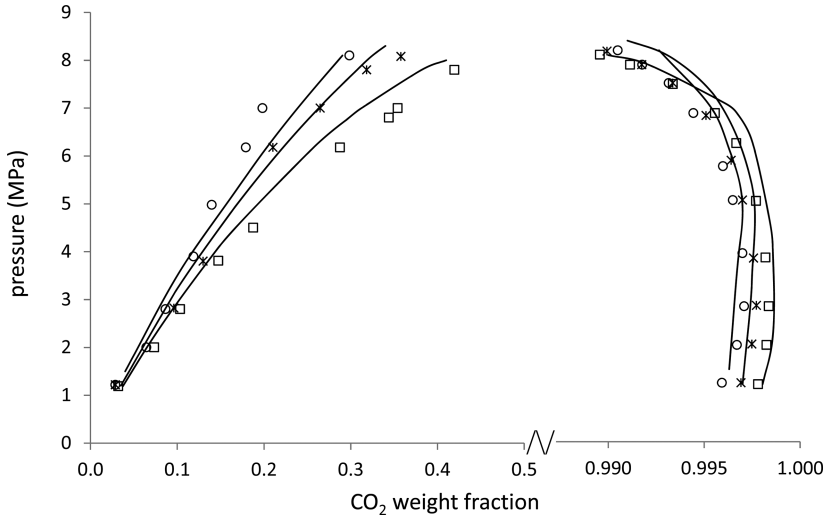


Figure 1. Vapor−liquid equilibria representation of the binary system ethyl lactate + CO_2: □, 311 K; ∗, 318 K and ○, 323 K. Liquid-phase composition: this work; vapor-phase composition: Chylinski et al.;[12] —, GC-EoS best fit (set 2 of groups to represent ethyl lactate molecule).

dx.doi.org/10.1021/je301039e | J. Chem. Eng. Data 2013, 58, 301−306

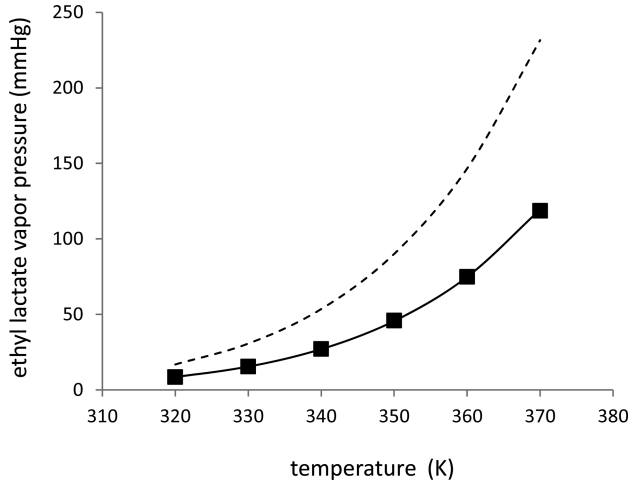


Figure 3. Calculation of ethyl lactate vapor pressure using the GC-EoS model: ■, experimental data;[19] - - -, GC-EoS prediction using current group matrix (set 1 of groups); —, GC-EoS fitting using a new alcohol−ester group to represent ethyl lactate group composition (set 2 of groups).

ethyl lactate vapor pressures. The parameters obtained from the regression procedure are given in Table 2. An excellent representation of ethyl lactate vapor pressure can be achieved (AAD = 0.39 %) when set 2 of groups is applied (Figure 3).

Further, when using set 1 of groups to describe the ethyl lactate molecule, high deviations were obtained also in the calculation of phase equilibrium compositions. The absolute average deviations in the liquid and vapor phases:

Table 2. GC-EoS Parameters Utilized To Calculate Vapor Pressure Data of Ethyl Lactate and Vapor−Liquid Phase Equilibria Compositions of the Binary Mixture Ethyl Lactate + CO_2

Pure Group Parameters					
	reference temperature	group surface area	pure group energy parameters		
	T^*	q	g	g'	g''
CH_3	600	0.848	316910.	−0.9274	0.0
CH_2	600	0.540	356080.	−0.8755	0.0
CH_2COO	600	1.420	831400.	−1.0930	0.0
CHOH	512.6	0.908	1207500.	−0.6444	0.0
CHOHCOO[a]	600	1.788	891900.	−1.0993	0.0
CO_2	304.2	1.261	531890.	−0.5780	0.0
Binary Group Interaction Parameters					
		attractive energy parameters		nonrandomness parameters	
i	j	k_{ij}	k'_{ij}	α_{ij}	α_{ji}
CHOHCOO[a]	CH_3/CH_2	1.054	0.018	1.777	1.777
	CO_2	1.076	−0.109	−2.126	−0.569
CO_2	CH_3	0.898	0.0	4.683	4.683
	CH_2	0.874	0.0	4.683	4.683
	CH_2COO	1.115	0.094	−1.615	−1.615
	CHOH	0.985	0.0	0.468	−0.390
CH_2COO	CH_3	0.869	0.0	0.0	0.0
	CHOH	0.996	−0.163	0.654	−2.612
CHOH	CH_3	0.715	0.0	10.220	1.471

[a]Parameters regressed in this work.

$$\mathrm{AAD} = \frac{1}{N}\sum\left(\frac{z_{\mathrm{exp}} - z_{\mathrm{cal}}}{z_{\mathrm{exp}}}\right) \quad (7)$$

were 16.0 % for z being the CO_2 weight fraction in the liquid phase ($w^{\mathrm{liq}}_{CO_2}$) and 96.7 % for the solubility of ethyl lactate (EL) in the vapor CO_2-rich phase ($z = w^{\mathrm{vap}}_{EL}$). Optimization of the CHOHCOO−CO_2 interaction parameters (see Table 2) allowed an accurate representation of the vapor−liquid phase compositions (see Figure 1) with AAD values of 8.2 % and 17.3 % for, respectively, the CO_2 weight fraction in the liquid phase and the ethyl lactate weight fraction in the vapor phase.

Phase Equilibria Modeling Using the SRK-EoS. The system that we focus on involves a medium-sized solute (ethyl lactate molar mass is 118). The normal boiling temperature of ethyl lactate was experimentally measured and reported (427.65 K).[20] To apply the EoS-based model, values for ethyl lactate critical properties are required. Nevertheless, those have not been measured experimentally and hence have to be estimated.

We apply the methods suggested by Wakeham et al.[21] and Brauner et al.[22] to estimate the critical properties of ethyl lactate, and the corresponding values obtained are T_c = 506.01 K, p_c = 28.22 bar, and ω = 0.5809.

It should be noted that the inaccuracy in the properties predictions plays a major role in the quality of the phase behavior calculations. In our case, as in many similar cases when there are no experimental data available with which to compare, it is recommendable to apply the generalized semitheoretical expression introduced by Zbogar et al.,[23] namely:

$$T_c/p_c = 9.0673 + 0.43309(Q_w^{\,1.3} + Q_w^{\,1.95}) \quad (8)$$

as a test of the reliability of the critical properties values estimated.

In the above, T_c is in Kelvin, p_c is in bar, and Q_w, which is a measure of the van der Waals molecular surface area, is a dimensionless parameter. Q_w is calculated as the sum of the group area parameters, Q_k:

$$Q_w = \sum_k \nu_k Q_k \quad (9)$$

where ν_k is the number of times group k appears in the molecule. The group area parameters Q_k are available in the universal functional activity coefficient (UNIFAC) tables.

The ratio of the ethyl lactate values estimated by us gives a very good approximation to the theoretically calculated T_c/p_c ratio, namely, 21.119 vs 20.457, respectively.

Another issue of importance, as discussed in detail by Fornari et al.,[24] is the influence of the mixing rule on the performance of the SRK-EoS. In the present case we have employed the 2PVDW mixing rule (eqs 4 and 5). The unlike-pair interaction parameters, k_{ij} and l_{ij}, were correlated using the experimental data measured in this study at the three temperatures of interest, applying a standard optimization procedure. The values obtained are shown in Table 3.

Figure 4 shows the quality of the vapor and liquid compositions representation achieved. AAD values (see eq 7) were 1.9 % and 6.6 % for, respectively, the CO_2 weight fraction in the liquid phase and the ethyl lactate weight fraction in the vapor phase.

Table 3. SRK-EoS Binary Prameters (k_{ij} and l_{ij}) Regressed to the Vapor−Liquid Phase Equilibria Data of the Binary Mixture Ethyl Lactate + CO_2

T/K	k_{ij}	l_{ij}
311	0.0152	0.0305
318	0.0229	0.0332
323	0.0257	0.0378

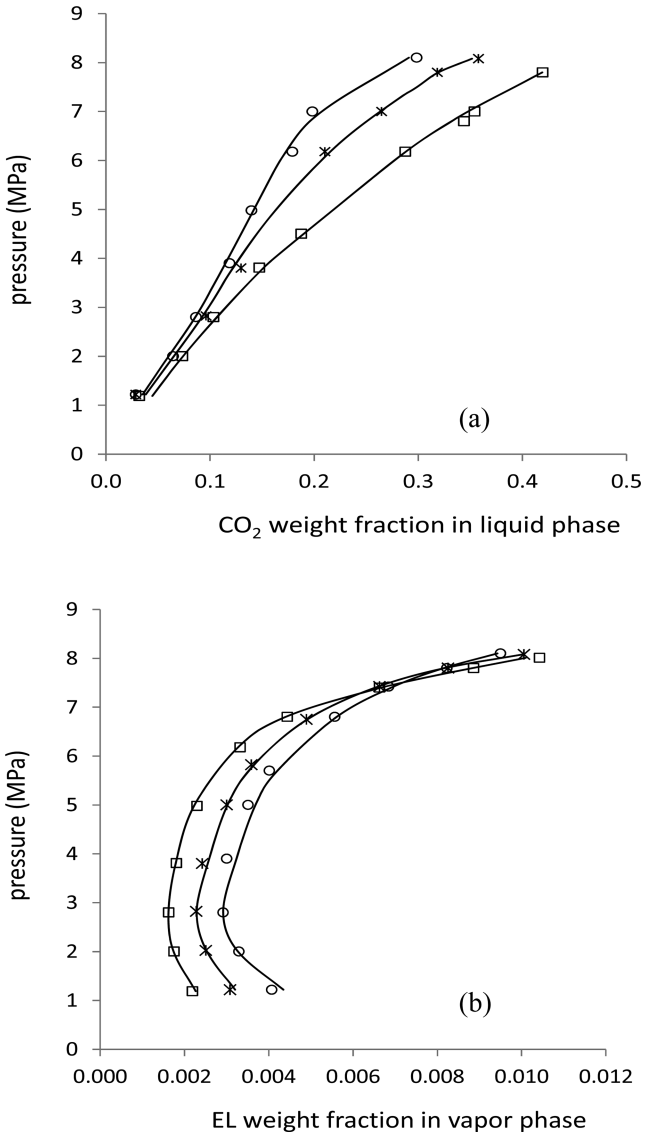


Figure 4. Vapor−liquid equilibria of the binary system ethyl lactate + CO_2. Symbols represent the experimental data, lines represent the SRK EoS correlation. □, 311 K; ∗, 318 K and ○, 323 K. (a) CO_2 weight fraction in liquid phase; (b) ethyl lactate weight fraction in vapor phase.

CONCLUSIONS

New data on the solubility of CO_2 in the ethyl lactate rich liquid phase of the binary system ethyl lactate + CO_2 at T = (311, 318, and 323) K and pressures ranging from (1 to 8.1) MPa is reported here for the first time. The capabilities of two different thermodynamic models to predict or correlate the phase behavior of the ethyl lactate + CO_2 mixture at the temperatures of interest to the experiment were tested. The results obtained were compared with our experimental data on the liquid phase and with the data on the vapor phase previously reported,[12] of the binary system studied.

The GC-EoS with the current parameter table[18] could provide neither a satisfactory representation of ethyl lactate vapor pressure nor of ethyl lactate + CO_2 vapor−liquid equilibria. This fact was attributed to the use of a non-appropriate ester group, since ethyl lactate is the derived ester of lactic acid. By defining a new alcohol−ester functional group (CHOHCOO) and fitting the corresponding parameters, a satisfactory representation was achieved with AAD of 0.39 % in the calculation of ethyl lactate vapor pressure, and 8.2 % and 17.3 % for, respectively, the CO_2 weight fraction in the liquid phase and the ethyl lactate weight fraction in the vapor phase.

The SRK-EoS with the 2PVDW mixing rule shows high correlative competence, with absolute average deviations lower than 7 % to represent both vapor- and liquid-phase compositions.

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Funding

The authors gratefully acknowledge the financial support from the Ministerio de Ciencia e Innovación (project 25506 FUN-C-FOOD) and Comunidad Autónoma de Madrid (ALIBIRD, project S-505/AGR-0153). D.V. is thankful for the JAE-pre fellowship given by Consejo Superior de Investigaciones Científicas (CSIC) of Spain.

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Weis, D. C.; Visco, D. P. Computer-aided molecular design using the Signature molecular descriptor: Application to solvent selection. *Comput. Chem. Eng.* **2010**, *34*, 1018−1029.

(2) Guo, H.; Wang, W.; Sun, Y.; Li, H.; Ai, F.; Xie, L.; Wang, X. Ethyl lactate enhances ethylenediaminedisuccinic acid solution removal of copper from contaminated soils. *J. Hazard. Mater.* **2010**, *174*, 59−63.

(3) Muse, J.; Colvin, H. A. U.S. Patent, US2005/0287179 A1, 2005.

(4) McConville, J. T.; Carvalho, T. C.; Kucera, S. A.; Garza, E. Ethyl lactate as a pharmaceutical-grade excipient and development of a sensitive perodixe assay. *Pharm. Technol.* **2009**, *33*, 74−82.

(5) Ishida, B. K.; Chapman, M. H. Carotenoid extraction from plants using a novel, environmentally friendly solvent. *J. Agric. Food Chem.* **2009**, *57*, 1051−1059.

(6) Strati, I. F.; Oreopoulou, V. Effect of extraction parameters on the carotenoid recovery from tomato waste. *Int. J. Food Sci. Technol.* **2011**, *46*, 23−29.

(7) Tombokan, X. C.; Aguda, R. M.; Danehower, D. A.; Kilpatrick, P. K.; Carbonell, R. G. Three-component phase behavior of the sclareol-ethyl lactate-carbon dioxide system for GAS applications. *J. Supercrit. Fluids* **2008**, *45*, 146−155.

(8) Golmakani, M.-T.; Mendiola, J. A.; Rezaeic, K.; Ibáñez, E. Expanded ethanol with CO_2 and pressurized ethyl lactate to obtain fractions enriched in γ-Linolenic Acid from Arthrospira platensis (Spirulina). *J. Supercrit. Fluids* **2012**, *62*, 109−115.

(9) Hernández, E. J.; Luna, P.; Stateva, R. P.; Najdanovic-Visak, V.; Reglero, G.; Fornari, T. Liquid-liquid phase transition of mixtures comprising squalene, olive oil, and ethyl lactate: application to recover squalene from oil deodorizer distillates. *J. Chem. Eng. Data* **2011**, *56*, 2148−2152.

(10) Vicente, G.; Paiva, A.; Fornari, T.; Najdanovic-Visak, V. Liquid−liquid equilibria for separation of tocopherol from olive oil using ethyl lactate. *Chem. Eng. J.* **2011**, *172*, 879−884.

(11) Manic, M. S.; Villanueva, D.; Fornari, T.; Queimada, A. J.; Macedo, A. E.; Najdanovic−Visak, V. Solubility of high-value compounds in ethyl lactate: Measurements and modeling. *J. Chem. Thermodyn.* **2012**, *48*, 93−100.
(12) Chylinski, K.; Gregorowicz, J. Solubilities of (1-hexanol, or 1,2-hexanediol, or 2-hydroxypropanoic acid ethyl ester, or 2-hydroxyhexanoic acid ethyl ester) in supercritical CO_2. *J. Chem. Thermodyn.* **1998**, *30*, 1131−1140.
(13) Sheldon, R. A. The E factor: fifteen years on. *Green Chem.* **2007**, *9*, 1273−1283.
(14) Skjold-Jørgensen, S. Gas solubility calculations. II. Application of a new group-contribution equation of state. *Fluid Phase Equilib.* **1984**, *16*, 317−351.
(15) Soave, G. Equilibrium constants from a modified Redlich-Kwong equation of state. *Chem. Eng. Sci.* **1972**, *27*, 1197−1203.
(16) Hernández, E. J.; Mabe, G. D.; Señoráns, F. J.; Reglero, G.; Fornari, T. High-Pressure Phase Equilibria of the Pseudoternary Mixture Sunflower Oil + Ethanol + Carbon Dioxide. *J. Chem. Eng. Data* **2008**, *53*, 2632−2636.
(17) Bondi, A. van der Waals volumes and radii. *J. Phys. Chem.* **1964**, *68*, 441−451.
(18) Fornari, T. Revision and summary of the group contribution equation of state parameter table: application to edible oil constituents. *Fluid Phase Equilib.* **2007**, *262*, 187−209.
(19) İnal, M. *Ethyl lactate production by hybrid processes: determination of phase diagrams and evaluation of performance of organophilic pervaporation membranes*. Master of Science Thesis, Middle East Technical University, Turkey, 2003.
(20) Yaws, C. L. *Chemical Properties Handbook*; McGraw-Hill: New York, 1999.
(21) Wakeham, W. A.; Cholakov, G.; Stateva, R. P. Liquid density and critical properties of hydrocarbons estimated from molecular structure. *J. Chem. Eng. Data* **2002**, *47*, 559−570.
(22) Brauner, N.; Stateva, R. P.; Cholakov, G.; Shacham, M. Structurally "targeted" quantitative structure−property relationship method for property prediction. *Ind. Eng. Chem. Res.* **2006**, *45*, 8430−8437.
(23) Zbogar, A.; Da Silva Lopes, F. V.; Kontogeorgis, G. M. Approach suitable for screening estimation methods for critical properties of heavy compounds. *Ind. Eng. Chem. Res.* **2006**, *45*, 476−480.
(24) Fornari, T.; Luna, P.; Stateva, R. P. The vdW EoS hundred years later, yet younger than before. Application to the phase equilibria modeling of food-type systems for a green technology. *J. Supercrit. Fluids* **2010**, *55*, 579−593.

3.2. Extracción de cafeína de matrices vegetales utilizando lactato de etilo

3.2.1. Extracción de cafeína de granos de café verde y hojas de té verde con lactato de etilo presurizado

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Extraction of caffeine from natural matter using a bio-renewable agrochemical solvent

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ABSTRACT

This paper reports experimental data on the pressurized liquid extraction of caffeine from green coffee beans and green tea leaves using ethyl lactate (ethyl 2-hydroxy-propanoate). This solvent is a new bio-renewable agrochemical solvent, naturally produced by fermentation from corn derived feedstock, which has been recently considered as a very suitable and environmental benign solvent for food industrial applications.

Static extraction assays (one step during 10 min) were carried out in an Accelerated solvent extraction (ASE) system at three different extraction temperatures, namely 100, 150 and 200 °C. Extraction yield and caffeine recovery were determined and compared with those obtained when using other liquid solvents, such as ethyl acetate or ethanol. High recovery of caffeine (≈60%) was found in the extracts produced using ethyl lactate, which demonstrates the potential use of this green solvent for the extraction of caffeine from different vegetable sources.

Keywords: Coffee; Green tea; Caffeine; Ethyl lactate; Accelerated solvent extraction

1. Introduction

Coffee plant belongs to the genus *Coffea* of the *Rubiaceae* family with more than 70 species, but only two of them have economic and commercial importance: the species Arabica (*Coffea arabica*) and Robusta (*Coffea robusta*) (Alonso-Salces et al., 2009). Arabica coffee beans are preferred by consumers and are considered of superior quality at the international market (Meinhart et al., 2010).

Coffee is one of the most traded commodities, and is one of the most popular drinks in the world due to its unique flavor and sensory characteristics. Coffee beans are an important source of caffeine, which is the most common consumed alkaloid in the world. Depending on coffee variety, caffeine content in green beans is around 1–2 %mass (Ashihara and Crozier, 2001). Other active principles present in coffee beans are coffee oil, an ingredient of special interest for the cosmetic and pharmaceutical industries (Folstar, 1985), and phenolic acids (elagic, caffeic and chlorogenic acids) to which several biological properties have been attributed (Naidu et al., 2008; Brezova et al., 2009).

Another plant that contains caffeine is green tea (*Camellia sinensis*), which has been a much consumed drink in Asian countries over years. Nowadays, it is very popular all over the world, due to its recognized beneficial health effects. Green tea leaves contains caffeine, catechins, fats, amino acids, aroma chemical, vitamins and chlorophyll, among others (Stone et al., 1991). Indeed, the major bioactive components are caffeine and catechins. Caffeine content is around 20–40 mg/g while catechins are in the range of 190–260 mg/g (Park et al., 2007a,b; Perva-Uzunalic et al., 2006). Catechins are recognized to be the beneficial bioactive compounds of green tea, including antioxidant, anticancer, anti-inflammatory, antibiotic and antiviral effects (Cai et al., 2002; Tedeschi et al., 2002; Cooper et al., 2005). Thus, the consumption of green tea is considerably increasing, so as products with green tea flavor such as beverages and ice-creams.

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Received 20 August 2012; Received in revised form 26 October 2012; Accepted 14 November 2012
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http://dx.doi.org/10.1016/j.fbp.2012.11.007

Unquestionably, supercritical CO_2 ($SCCO_2$) extraction has proved to be the most convenient commercial and environmental friendly technology for the removal of caffeine from green coffee beans (Zosel, 1978). $SCCO_2$ is selective for the caffeine, there is no associated waste treatment of a toxic solvent and extraction times are generally moderate. $SCCO_2$ coupled with a cosolvent, such as ethanol or water, was employed to extract caffeine form green tea leaves. By varying the extraction conditions, it is expected to maximize the amount of caffeine extracted and minimize the co-extraction of bioactive catechins. Nevertheless, substantial losses of catechins proved to be unavoidable (Kim et al., 2008; Park et al., 2007a,b).

Among liquid solvents traditionally employed for coffee decaffeination, benzene, chloroform, trichloroethylene and dichloromethane have been used over years. However, when evidence suggested that chlorinated solvents might be carcinogenic (Lynge et al., 1997) their use was severely reduced.

Extraction with water (definitely a green solvent) is also called "indirect extraction" since a two-step process is required: coffee beans are first soaked in water, an organic solvent is employed to selectively extract caffeine from water, and the caffeine-free water goes back in contact with the beans and is evaporated (Clarke, 2003). This procedure strips away many of the essential flavor and aroma substances. Ethyl acetate is much more selective for caffeine and thus, extraction can be accomplished in a single-step contact process ("direct extraction"). Since ethyl acetate presents much less health and environmental hazard than chlorinated solvents, decaffeination of coffee beans using this solvent is often called "natural decaffeination" despite the fact that the ethyl acetate employed was obtained from synthesis and not from natural sources.

As green coffee beans, the current commercially available methods for decaffeinating green tea leaves have been solvent based extraction, using chlorinated solvents, ethyl acetate, acetone, methanol, ethanol and acetonitrile (Senol and Aydin, 2006). Effective decaffeination can be achieved using these solvents but catechins are also significantly co-extracted, reducing the value of green tea as a functional healthy drink.

Ethyl lactate (ethyl 2-hydroxy-propanoate) is an agrochemical and economically viable alternative to traditional liquid solvents, and it is fully biodegradable, non-corrosive, non-carcinogenic and non-ozone depleting. It was self-affirmed GRAS (generally recognized as safe) and due to its low toxicity, was approved by the U.S. Food and Drug Administration (FDA) as pharmaceutical and food additive. These characteristics have increased the attention to the use of ethyl lactate as a green solvent for the food industry. Several reported potential applications are related with the extraction of carotenoids from different plant matrix (Ishida and Chapman, 2009; Strati and Oreopoulou, 2011), the extraction of γ-linolenic acid from Spirulina (Golmakani et al., 2012) and with the fractionation of edible oil compounds (squalene and tocopherol) (Hernández et al., 2011; Vicente et al., 2011).

The solubility of caffeine in ethyl lactate has been reported by the authors in a recent contribution (Manic et al., 2012). At 303 K the solubility was reported to be 3.2% by mass, which is very similar to the values reported for the solubility of caffeine in water (Bustamante et al., 2002). These solubility data motivated the present study: the potential use of ethyl lactate as an environmentally friendly solvent to extract caffeine from natural matter. To the best of our knowledge the extraction of green coffee beans and green tea leaves using ethyl lactate is presented for the first time.

2. Materials and methods

2.1. Samples and reagents

Green coffee beans (*C. arabica* variety) and green tea (*C. sinensis*) leaves were acquired in a Spanish market. Water content in beans and leaves was determined to be, respectively, 11.2% and 6.2%. The moisture content was determined by oven drying at 80 °C until a constant weight was obtained.

The coffee beans were ground in a ceramic mortar using liquid nitrogen; particle sizes were separated by using sieves with manual agitation. Two different sizes of particles were employed in the experiments: the entire green beans and ground beans with particles in the range of 500–1500 μm. The green tea leaves were ground in a manual knife mill using liquid nitrogen; particle of sizes 200–500 μm were employed for the experiments and were separated by using sieves with manual agitation.

Ethyl lactate (≥98% purity) and ethyl acetate (≥99.7% purity) were obtained from Sigma–Aldrich (St. Louis, MO, USA), and ethanol (99.5% (v/v) purity) from Panreac (Castellar del Vallés, Barcelona, Spain). Caffeine standard (≥99.0% purity) was obtained from Fluka (Höchstädtan der Donau, Germany).

2.2. Accelerated solvent extraction (ASE)

Caffeine extraction was carried out in an Accelerated Solvent Extraction System ASE 350 from Dionex Corporation (Sunnyvale, CA, USA) equipped with a solvent controller unit. A scheme of the equipment is shown in Fig. 1.

In the case of coffee beans samples, extractions were performed with three different liquid solvents (i.e. ethyl lactate, ethanol and ethyl acetate) at three different extraction temperatures (100, 150 and 200 °C) using 3 g of solid sample. The extraction of caffeine from green tea leaves was performed at 100, 150 and 200 °C, with ethyl lactate and ethanol as extractive solvents. In this case, each cell was filled with around 1 g of solid sample.

Several preliminary assays were carried out in order to analyze the effect of time in the extraction procedure. At 200 °C, very similar yields were obtained employing 10 min and 20 min of static extraction, being the differences lower than 15%. In view of this, and in order to reduce the possibility of thermal degradation, a static extraction time of 10 min was selected to carry out all experiments.

The experimental procedure for both raw materials was as follows. The cells employed (10 mL capacity) were placed into an oven; each cell was filled with the corresponding amount of solid sample. After loading the sample into the extraction cell, the cell was filled with the corresponding solvent up to a pressure of 10 MPa (which ensures the liquid state of the three solvents employed at the three temperatures studied) and was heated-up to the desired temperature. Then, a static extraction continued for 10 min with all the system valves closed. In order to prevent over-pressurization of the cell, a static valve was pulsed open and closed automatically when the cell pressure exceeded the set point. The solvent that escaped during this venting was collected in the collection vial. After extraction the cell was washed with the solvent and subsequently

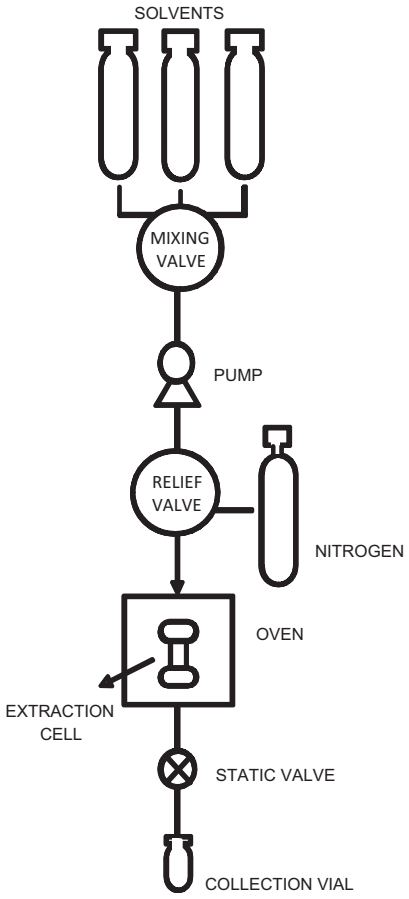

Fig. 1 – Scheme of ASE device employed in the extraction of caffeine from green coffee beans and green tea leaves.

the solvent was purged from cell using N_2 gas until complete depressurization was accomplished.

The extracts were stored under refrigeration until they were dried. A rotavapor was used for partial elimination of solvent. Then, a thermo-block at 100 °C was used to dry the samples to a constant weight. All experiments were carried out by duplicate. The dried samples obtained were stored at 4 °C until analysis.

2.3. *Identification and quantification of caffeine*

A Varian ProStar Analytical HPLC (Agilent Technologies, Santa Clara, CA, USA) with a ternary pump to create gradients, thermostatic controlled column oven, autosampler mode 410 with a 20 μL sample loop and diode array detector was employed in the study. All the modules were controlled by PC with interface and HPLC Varian Star system control software.

The column employed was Microsorb-MV100 column C-18, 5 μm (250 mm × 4.6 mm), fitted with a suitable pre-column. Based in the method of Sharma et al. (2005) the mobile phase adopted was (A) acetonitrile/(B) 0.1% mass ortho-phosphoric acid in water with a flow rate of 0.8 mL/min and column compartment temperature of 35 °C. The mobile phase gradient employed was as follows: initial 10% A, 15 min 30% A, 20 min 35% A, 22 min 20% A and 25 min 10% A. The amount of caffeine in the different samples was calculated from a calibration curve of caffeine standard. HPLC analysis was carried out by duplicate.

2.4. *Determination of total caffeine content in green coffee beans*

The beans were ground in a coffee mill using liquid nitrogen and particles separated using sieves and manual agitation. In this case, particles of small size (250–500 μm) were selected in order to enhance the extraction of caffeine from solid matrix. Then, 3 g of sample was extracted with ethanol at 60 °C in a Stuart Orbital S150 shaker apparatus (Bibby Scientific Limited, Stone, UK) during 80 h. Solvent was renewed at different intervals of time, and the extraction was finished when the caffeine extracted in the corresponding interval of time was around 2% of total caffeine extracted.

2.5. *Determination of other bioactive substances in coffee extracts*

2.5.1. *Coffee oil*

The content of lipid-type compounds present in the coffee beans extracts was obtained gravimetrically after hexane extraction. Base-catalyzed methanolysis (Vázquez et al., 2008) of these extracts was accomplished to determine the fatty acid profile. The GC–MS analyses (Agilent 7890A System – Agilent Technologies, Santa Clara, CA, USA) were based on the method reported by Lu et al. (2004). The main FAMEs present in the sample were identified by comparison with standard mass spectra from library (Wiley 229).

2.5.2. *Total phenolic compounds (TPCs)*

The presence of phenolic-type antioxidants was determined using the Folin–Ciocalteu reagent by the Singleton et al. (1999) method. The results were expressed as GAE (mg of gallic acid/g of sample). 3 mL of distilled water was mixed with 50 μL of sample or standard. Then, 250 μL of Folin–Ciocalteu reagent was added and the content of the tube was mixed thoroughly. After 3 min, 0.75 mL of Na_2CO_3 (20 %mass) followed by 0.95 mL of distilled water was added and the mixture was allowed to stand for 2 h. The absorbance was measured at 760 nm.

3. Results and discussion

3.1. *Extraction of caffeine from natural matter*

Fig. 2 shows the kinetic behavior of caffeine extraction from grounded coffee beans during 80 h of extraction in the Stuart Orbital S150 shaker apparatus, and renewing the solvent (ethanol) at different intervals of time. Taking into account these results, the content of caffeine in the green coffee beans was estimated to be 9.3 mg of caffeine/g coffee beans, which compares reasonably with the values reported for *C. arabica* variety in the literature (Ashihara and Crozier, 2001) (caffeine content in green beans ≈1 %mass).

Table 1 shows the yields obtained in the extraction of the entire and ground green coffee beans, using three different solvents, namely ethyl lactate, ethanol and ethyl acetate, at 100, 150 and 200 °C. The caffeine concentration is also reported in the table for all samples collected. The average relative standard deviations of the *N* experiments (ARSD):

$$\mathrm{ARSD} = \frac{1}{N}\sum \frac{\mathrm{SD}_i}{\bar{x}_i} \quad (1)$$

were 9.4% and 7.7%, respectively, for extraction yield and caffeine content in the samples.

Table 1 – Extraction yield (g of extract/g of beans × 100), caffeine content (g caffeine/g extract × 100) and caffeine recovery (mg of caffeine/g of beans) obtained in the ASE of green coffee beans using ethyl lactate, ethanol and ethyl acetate.

		Extraction temperature		
		100 °C	150 °C	200 °C
Solvent: ethyl lactate				
Entire beans	Extraction yield	0.31 ± 0.06	1.48 ± 0.02	13 ± 3
	Caffeine content	2.6 ± 0.6	5.3 ± 0.5	4.3 ± 0.3
	Caffeine recovery	0.08	0.78	5.36
Ground beans	Extraction yield	1.79 ± 0.03	2.75 ± 0.05	10.1 ± 0.1
	Caffeine content	3.86 ± 0.03	6.2 ± 0.2	5.8 ± 0.2
	Caffeine recovery	0.69	1.71	5.87
Solvent: ethanol				
Entire beans	Extraction yield	0.4 ± 0.2	1.41 ± 0.04	5.7 ± 0.6
	Caffeine content	5 ± 2	7.5 ± 0.3	7.8 ± 0.5
	Caffeine recovery	0.21	1.06	4.40
Ground beans	Extraction yield	2.4 ± 0.1	3.6 ± 0.1	8.7 ± 0.3
	Caffeine content	4.8 ± 0.4	7.29 ± 0.01	6.8 ± 0.2
	Caffeine recovery	1.16	2.59	5.93
Solvent: ethyl acetate				
Entire beans	Extraction yield	0.23 ± 0.03	0.38 ± 0.07	3.3 ± 0.3
	Caffeine content	6.8 ± 0.2	16 ± 2	11.8 ± 0.2
	Caffeine recovery	0.16	0.59	3.83
Ground beans	Extraction yield	1.57 ± 0.08	1.97 ± 0.07	4.5 ± 0.2
	Caffeine content	6.0 ± 0.4	10.25 ± 0.07	10.2 ± 0.2
	Caffeine recovery	0.94	2.02	4.54

As expected, despite the solvent employed, extraction yield increased considerably with temperature. Further, the most significant increase of extraction yield with temperature was observed in the case of using ethyl lactate. In general, higher extraction yields were obtained when processing the ground beans than when employing the entire beans, although in the case of ethyl lactate at 200 °C differences were not noteworthy.

Despite the solvent employed, the higher concentrations (%mass of caffeine in the extract) were obtained at 150 °C. That is, although a narrow range of temperatures were explored, and for all solvents studied, it appears that the selectivity toward the extraction of caffeine increased with temperature up to a maximum and then decreased. Certainly, the higher concentrations of caffeine in the extracts were obtained with ethyl acetate, followed by ethanol and ethyl lactate. That is, among the solvents employed, ethyl acetate is definitely the most selective to extract caffeine from coffee beans.

Considering the value obtained for the content of caffeine in Arabica green coffee beans (9.3 mg/g beans) (Ashihara and Crozier, 2001), it can be concluded that high caffeine recovery was obtained (60%) using ethyl lactate at 200 °C. These values are 30–40% higher in comparison to the values obtained with ethyl acetate, which is considered the greenest solvent for direct coffee decaffeination. Thus, ethyl lactate may be a viable ecological alternative liquid solvent for the extraction of caffeine from green coffee beans.

In order to test the capability of ethyl lactate to extract caffeine from other type of vegetable matter, the ASE extraction of green tea leaves was also accomplished. Table 2 shows the extraction yield, caffeine concentration and caffeine recovery obtained using ethyl lactate and ethanol solvents. Values reported for extraction yield and caffeine content are the average values obtained from duplicate experiments; the ARSD (see Eq. (1)) were, respectively, 4.3% and 3.6%.

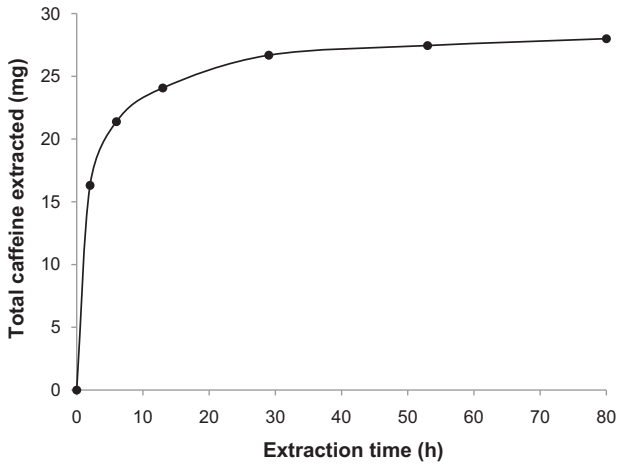

Fig. 2 – Total caffeine recovered in 3 g of green coffee beans: kinetic behavior of caffeine extraction using ethanol at 60 °C (ambient pressure) in a Stuart Orbital shaker.

Table 2 – Extraction yield (g of extract/g of tea leaves × 100), caffeine content (g caffeine/g extract × 100) and caffeine recovery (mg of caffeine/g of tea leaves) obtained in the ASE of green tea leaves using ethyl lactate and ethanol.

	Extraction temperature		
	100 °C	150 °C	200 °C
Solvent: ethyl lactate			
Extraction yield	14.3 ± 0.6	26 ± 3	50 ± 1
Caffeine content	8.4 ± 0.3	7.2 ± 0.5	4.5 ± 0.2
Caffeine recovery	11.98	18.92	22.58
Solvent: ethanol			
Extraction yield	19.0 ± 0.5	29.0 ± 0.2	40 ± 2
Caffeine content	10.3 ± 0.3	8.1 ± 0.1	5.9 ± 0.2
Caffeine recovery	19.62	23.45	23.91

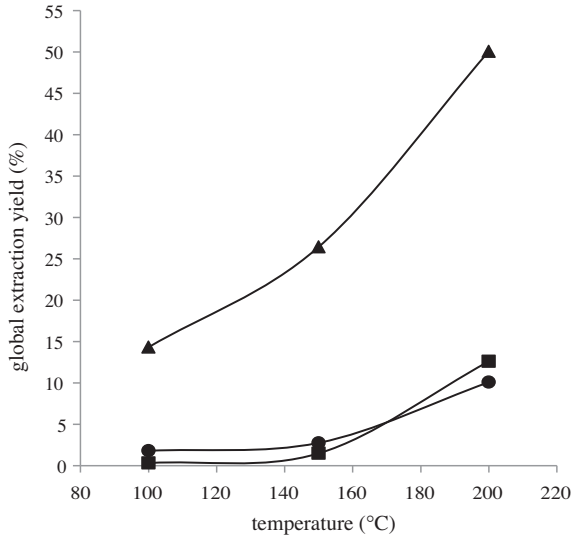

Fig. 3 – Extraction yield obtained in the ASE extraction of green coffee beans and green tea leaves using ethyl lactate. (■) entire coffee beans; (●) ground coffee beans; and (▲) tea leaves.

Both solvents employed exhibit similar behavior regarding the extraction of caffeine. As expected, extraction yield increased considerably with temperature, with a remarkable increase at 200 °C in the case of ethyl lactate particularly in the extraction of green coffee beans. As can be observed in Fig. 3, the increases observed for entire and ground beans samples are, respectively, 4.8% and 1.5% when temperature increase from 100 °C to 150 °C, while these values are 8.5% and 3.7% when temperature increase from 150 °C to 200 °C. That is, a significant increase of the solvent power of ethyl lactate is observed when the extraction temperature became higher than normal boiling point of the solvent (154 °C).

The concentration of caffeine in the green tea extracts decreased with increasing temperature, demonstrating that the removal of caffeine from the green tea leaves is much selective at the lower temperature investigated (100 °C). Further, according to the reported values for the content of caffeine in green tea leaves (20–40 mg/g) (Park et al., 2007a,b; Perva-Uzunalic et al., 2006) it appears that the ASE using ethyl lactate may provide high removal of caffeine also from green tea leaves.

Table 3 – Total phenolic compounds (TPCs) in green coffee beans extracts measured using the Folin–Ciocalteu reagent (g of gallic acid equivalents/g of extract × 100). ARSD (average relative standard deviation) = 4.12%.

	Extraction temperature		
	100 °C	150 °C	200 °C
Ethyl lactate			
Entire beans	2.51	5.31	8.67
Ground beans	10.64	11.77	12.67
Ethanol			
Entire beans	5.84	5.87	13.94
Ground beans	8.97	9.84	13.91
Ethyl acetate			
Entire beans	5.32	8.94	17.44
Ground beans	3.07	5.71	15.74

Table 4 – Content of lipid-type compounds (LTC) (g oil/g extract × 100) obtained in the green coffee beans extracts using ethyl lactate, ethanol and ethyl acetate. ARSD (average relative standard deviation) = 7.71%.

	Extraction temperature		
	100 °C	150 °C	200 °C
Ethyl lactate			
Entire beans	12.9	3.2	4.7
Ground beans	48.4	36.9	10.4
Ethanol			
Entire beans	17.4	8.8	6.6
Ground beans	41.8	31.2	25.6
Ethyl acetate			
Entire beans	16.0	17.9	13.6
Ground beans	79.7	60.6	29.0

3.2. *Analysis of the co-extraction of other bioactive substances from green coffee beans*

As mentioned before, coffee oil and phenolic compounds (mainly chlorogenic acids) are also present in coffee beans and are valuable components of coffee due to their positive biological activity. Further, these substances have an important role during coffee roasting since the high temperatures provoke their transformation into key compounds of coffee flavor and aroma (Farah et al., 2006). Thus, it is desirable to reduce the removal of phenolic compounds and coffee oil during decaffeination.

The co-extraction of these compounds is described in Tables 3 and 4, where is reported the content (%mass) of total phenolic compounds (TPCs) and lipid-type compounds (LTCs) determined in the extracts. By increasing the extraction temperature, increasing concentrations of TPC were found in the samples (particularly in the case of ground coffee beans) while decreasing concentrations of LTC were determined. Further,

Table 5 – Recovery[a] of total phenolic compounds (TPC) and lipid-type compounds (LTC) (g extracted/g of beans × 100) in the ASE of green coffee beans using ethyl lactate, ethanol and ethyl acetate.

		Extraction temperature		
		100 °C	150 °C	200 °C
Solvent: ethyl lactate				
Entire beans	TPC	0.2	1.7	23.7
	LTC	0.4	0.4	5.5
Ground beans	TPC	4.1	7.0	27.8
	LTC	8.0	9.4	9.7
Solvent: ethanol				
Entire beans	TPC	0.6	1.8	17.1
	LTC	0.7	1.1	3.5
Ground beans	TPC	4.8	7.6	26.2
	LTC	9.4	10.3	20.6
Solvent: ethyl acetate				
Entire beans	TPC	0.3	0.7	12.3
	LTC	0.3	0.6	4.1
Ground beans	TPC	1.0	2.4	15.3
	LTC	11.6	11.1	12.0

[a] TPC and LTC present in raw material were taken from the literature (Alonso-Salces et al., 2009; Oliveira et al., 2006).

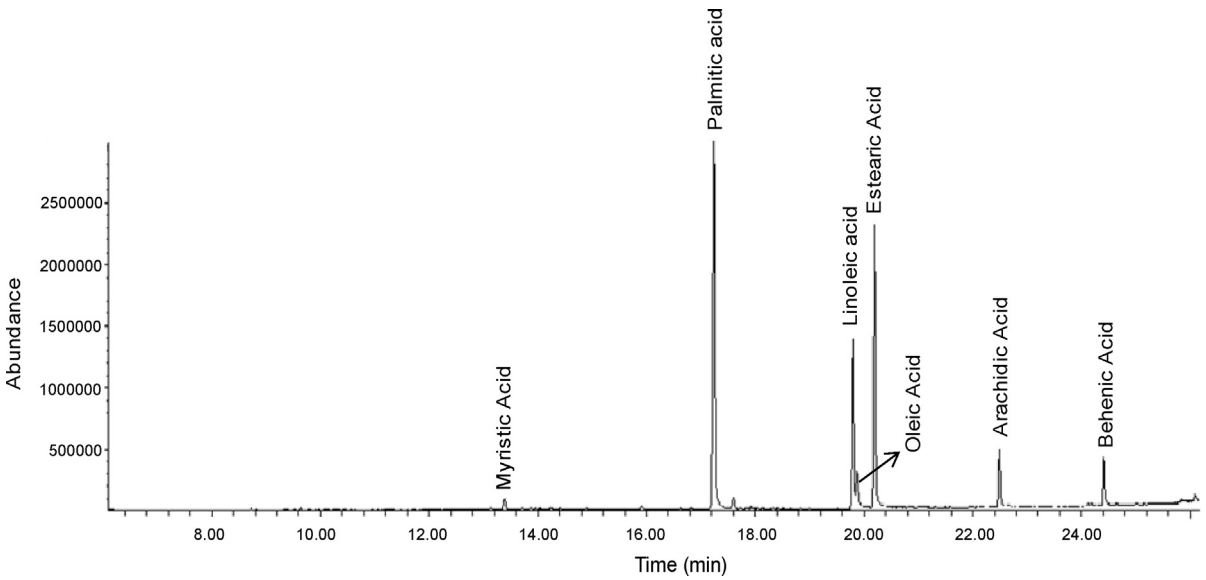

Fig. 4 – Fatty acid profile obtained by GC analysis of green coffee beans (entire samples) ASE extracts obtained at 100 °C, 10 MPa and using ethyl acetate.

considerably higher concentrations of LTC were extracted in the case of ground beans in comparison with entire beans. The main fatty acids identified in the samples were palmitic, linoleic and stearic acids (see Fig. 4) in accordance with the literature (Dussert et al., 2008).

Taking into consideration the mean values reported in the literature for the content of phenolic acids and coffee oil in green coffee beans, that is, respectively, 46 mg (Alonso-Salces et al., 2009) and 108 mg (Oliveira et al., 2006) per gram of beans, the co-extraction of phenolic and lipid compounds was assessed and compared. Table 5 shows the recovery of TPC and LTC calculated for the different extracts obtained. For all solvents studied, including ethyl lactate, the co-extraction of phenolic compounds and coffee oil represent, respectively, less than 28% and 21% of the corresponding amounts present in the raw material. Particularly, ethyl lactate behavior is quite similar to that of ethanol. Further, ethyl lactate extracted similar amounts of coffee oil but almost twice amounts of phenolic compounds in comparison with ethyl acetate. These results may be attributed to the higher polarity of ethyl lactate in comparison with ethyl acetate, due to the hydroxyl group present in its chemical structure.

4. Conclusions

The potential use of ethyl lactate in the extraction of caffeine from natural matter, namely green coffee beans and green tea leaves, was presented in this work. Accelerated solvent extraction (ASE) of green coffee beans at 200 °C provided higher caffeine recovery using ethyl lactate than when using ethyl acetate. Further, also high caffeine recoveries were obtained in the ASE of green tea leaves. Thus, ethyl lactate seems to be a good agrochemical solvent for the extraction of caffeine from vegetal sources.

In the case of the extraction of green coffee beans, preliminary study of the co-extraction of other important substances present in the natural matter also derived in encouraging the potential use of ethyl lactate. The co-extraction of phenolic compounds and coffee oil was, respectively, lower than 28% and 10%. Additional studies are under development in order to determine optimal conditions to selectively extract caffeine form tea leaves, minimizing the co-extraction of catechin-type bioactive substances.

Acknowledgments

The authors gratefully acknowledge the financial support from the Ministerio de Ciencia e Innovación of Spain (joined project Spain-Portugal, PT2009-0010) and the financial support from the Conselho de Reitores das Universidades Portuguesas (CRUP) – Integrated project Portugal – Spain, No. E-95/10. P. Luna and D. Villanueva acknowledges, respectively, the post-doctoral and predoctoral contract (JAE) given by the Consejo Superior de Investigaciones Científicas (CSIC) of Spain. M.S. Manic is thankful to Fundação para a Ciência e Tecnologia – Portugal for a doctoral fellowship (SFRH/BD/45323/2008).

References

Alonso-Salces, R.M., Serra, F., Reniero, F., Herberger, K., 2009. Botanical and geographical characterization of green coffee (*Coffea arabica* and *Coffea canephora*): chemometric evaluation of phenolic and methylxanthine contents. J. Agric. Food Chem. 57, 4224–4235.

Ashihara, H., Crozier, A., 2001. Caffeine: a well known but little mentioned compound in plant science. Trends Plant Sci. 6, 407–413.

Brezova, V., Šlebodova, A., Staško, A., 2009. Coffee as a source of antioxidants: an EPR study. Food Chem. 114, 859–868.

Bustamante, P., Navarro, J., Romero, S., Escalera, B., 2002. Thermodynamic origin of the solubility profile of drugs showing one or two maxima against the polarity of aqueous and nonaqueous mixtures: niflumic acid and caffeine. J. Pharm. Sci. 91, 874–883.

Cai, Y.J., Ma, L.P., Hou, L.F., Zhou, B., Yang, L., Liu, Z.L., 2002. Antioxidant effects of green tea polyphenols on free radical initiated peroxidation of rat liver microsomes. Chem. Phys. Lipids 120, 109–117.

Clarke, R.J., 2003. Decaffeination. In: Trugo, L., Finglas, P.M. (Eds.), Encyclopedia of Food Sciences and Nutrition. , 2nd ed. Elsevier Applied Science Publishers Ltd., New York, pp. 1506–1511.

Cooper, R., Morre, D.J., Morre, D.M., 2005. Medicinal benefits of green tea: Part I. Review of non cancer health benefits. J. Altern. Complement. Med. 11, 521–528.

Dussert, S., Laffargue, A., de Kochko, A., Joët, T., 2008. Effectiveness of the fatty acid and sterol composition of seeds

for the chemotaxonomy of *Coffea* subgenus *Coffea*. Phytochemistry 69, 2950–2960.
Farah, A., Monteiro, M.C., Calado, V., Franca, A.S., Trugo, L.C., 2006. Correlation between cup quality and chemical attributes of Brazilian coffee. Food Chem. 98, 373–380.
Folstar, P., 1985. Lipids. In: Clarke, R.J., Macrae, R. (Eds.), Coffee: Chemistry, vol. 1. Elsevier Applied Science Publishers Ltd., New York, pp. 203–221.
Golmakani, M-T., Mendiola, J.A., Rezaeic, K., Ibáñez, E., 2012. Expanded ethanol with CO_2 and pressurized ethyl lactate to obtain fractions enriched in γ-linolenic acid from *Arthrospira platensis* (Spirulina). J. Supercrit. Fluids 62, 109–115.
Hernández, E.J., Luna, P., Stateva, R.P., Najdanovic-Visak, V., Reglero, G., Fornari, T., 2011. Liquid–liquid phase transition of mixtures comprising squalene, olive oil, and ethyl lactate: application to recover squalene from oil deodorizer distillates. J. Chem. Eng. Data 56, 2148–2152.
Ishida, B.K., Chapman, M.H., 2009. Carotenoid extraction from plants using a novel, environmentally friendly solvent. J. Agric. Food Chem. 57, 1051–1059.
Kim, W.J., Kim, J.D., Kim, J., Oh, S.G., Lee, Y.W., 2008. Selective caffeine removal from green tea using supercritical carbon dioxide extraction. J. Food Eng. 89, 303–309.
Lu, T., Chen, C., Yang, F., Fung, J., Chen, M., Tsay, S., Li, J., Zou, W., Wu, S., 2004. Structure of a major glycolipid from *Thermus oshimai* NTU-063. Carbohydr. Res. 339, 2593–2598.
Lynge, E., Anttila, A., Hemminki, K., 1997. Organic solvents and cancer. Cancer Causes Control 8, 406–419.
Manic, M.S., Villanueva, D., Fornari, T., Queimada, A.J., Macedo, E.A., Najdanovic-Visak, V., 2012. Solubilities of high-value compounds in ethyl lactate: measurements and modelling. J. Chem. Thermodyn. 48, 93–100.
Meinhart, A., Bizzotto, C., Ballus, C.A., Prado, M.A., Bruns, R.E., Filho, J.T., Godoy, H.T., 2010. Optimization of a CE method for caffeine analysis in decaffeinated coffee. Food Chem. 120, 1155–1161.
Naidu, M.M., Sulochanamma, G., Sampathu, S.R., Srinivas, P., 2008. Studies on extraction and antioxidant potential of green coffee. Food Chem. 107, 377–384.
Oliveira, L.S., Franca, A.S., Mendonça, J.C.F., Barros-Júnior, M.C., 2006. Proximate composition and fatty acids profile of green and roasted defective coffee beans. LWT: Food Sci. Technol. 39, 235–239.
Park, H.S., Choi, H.K., Lee, S.J., Park, K.W., Choi, S.G., Kim, K.H., 2007a. Effect of mass transfer on the removal of caffeine from green tea by supercritical carbon dioxide. J. Supercrit. Fluids 42, 205–212.
Park, H.S., Lee, H.J., Shin, M.H., Lee, K.W., Lee, H., Kim, Y.S., Kim, K.O., Kim, K.H., 2007b. Effects of cosolvents on the decaffeination of green tea by supercritical carbon dioxide. Food Chem. 105, 1011–1017.
Perva-Uzunalic, A., Skerget, M., Knez, Z., Weinreich, B., Otto, F., Gruner, S., 2006. Extraction of active ingredients from green tea (*Camellia sinensis*): extraction efficiency of major catechins and caffeine. Food Chem. 96, 597–605.
Senol, A., Aydin, A., 2006. Solid–liquid extraction of caffeine from tea waste using battery type extractor: process optimization. J. Food Eng. 75, 565–573.
Sharma, V., Gulati, A., Ravindranath, S.D., Kumar, V.A., 2005. Simple and convenient method for analysis of tea biochemicals by reverse phase HPLC. J. Food Compos. Anal. 18, 583–594.
Singleton, V.L., Orthofer, R., Lamuela-Raventós, R.M., 1999. Analysis of total phenols and other oxidations substrates and antioxidants by means of Folin–Ciocalteu reagent. Methods Enzymol. 299, 152–178.
Stone, M.H., Fleck, S.J., Kraemer, W.J., Triplett, N.T., 1991. Health and performance related changes adaptations to resistance training. Sports Med. 11, 210–231.
Strati, I.F., Oreopoulou, V., 2011. Effect of extraction parameters on the carotenoid recovery from tomato waste. Int. J. Food Sci.Technol. 46, 23–29.
Tedeschi, E., Suzuki, H., Menegazzi, M., 2002. Antiinflammatory action of EGCG the main component of green tea, through STAT-1 inhibition. Ann. N.Y. Acad. Sci. 973, 435–437.
Vázquez, L., Fornari, T., Señoráns, F.J., Reglero, G., Torres, C.F., 2008. Supercritical carbon dioxide fractionation of nonesterified alkoxyglycerols obtained from shark liver oil. J. Agric. Food Chem. 56, 1078–1083.
Vicente, G., Paiva, A., Fornari, T., Najdanovic-Visak, V., 2011. Liquid–liquid equilibria for separation of tocopherol from olive oil using ethyl lactate. Chem. Eng. J. 172, 879–884.
Zosel, K., 1978. Praktische Anwendungen der Stofftrennungmit Überkritischen Gasen. Chemie 90, 748–755.

Contents lists available at ScienceDirect

Food and Bioproducts Processing

journal homepage: www.elsevier.com/locate/fbp

Pressurized liquid extraction of caffeine and catechins from green tea leaves using ethyl lactate, water and ethyl lactate + water mixtures

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ARTICLE INFO

Article history:
Received 18 March 2015
Received in revised form 14 July 2015
Accepted 17 July 2015
Available online 26 July 2015

Keywords:
Green tea
Caffeine
Catechins
Ethyl lactate
Pressurized liquid extraction
Solubility

ABSTRACT

Ethyl-lactate (ethyl 2-hydroxy-propanoate) is a bio-renewable agrochemical solvent, very suitable and environmental benign for food applications, permitted by the U.S. Food and Drug Administration as pharmaceutical and food additive. In previous work, the authors demonstrated that pressurized liquid extraction (PLE) using ethyl lactate is a suitable alternative to remove caffeine from vegetal materials, e.g. green coffee beans and green tea leaves. The solubility of caffeine in ethyl lactate + water mixtures, at ambient temperature and pressure, exhibits a substantial increase for 60:40% ethyl lactate + water mixtures (data reported in this work). This result motivated the analysis of the effect of the ethyl lactate + water mixtures for the decaffeination target.

Furthermore, in the case of green tea, the removal of caffeine reducing the extraction of catechins is desirable due to the adverse effects of caffeine on health, while catechins are high valued functional food ingredients. Thus, the use of ethyl lactate, water and ethyl lactate + water mixtures to attain this objective, i.e. the removal of caffeine form green tea leaves minimizing the extraction of catechins, was studied in this work.

PLE was carried out in the temperature range 373–473 K and using different ethyl lactate + water mixtures. Extraction yield and recovery of key bioactive compounds (caffeine and monomeric catechins) were determined and compared, and the caffeine/catechins selectivity of the different solvents employed was estimated.

High extraction yields were obtained with a mixture containing 25:75% of ethyl lactate + water, with values around 1.5 and 3.5 times higher than, respectively, the yields obtained with water and ethyl lactate. Yet, pure ethyl lactate proved to be the most selective solvent to extract caffeine from green tea leaves, minimizing the co-extraction of catechins, with a caffeine/catechins selectivity of 2.8 to 5.5 in the range 373–423 K. At these temperatures, with short extraction times (20 min) the recovery of caffeine is in the range 53–76% but only 26–36% of catechins present in the tea leaves were removed.

1. Introduction

Tea is one of the most popular beverages in the world, is obtained from the leaves of the plant *Camellia sinensis* and green tea is one of the most widely consumed types. Green tea leaves contain several bioactive compounds, including methylxanthine alkaloids and polyphenols.

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http://dx.doi.org/10.1016/j.fbp.2015.07.008
0960-3085/

Caffeine is the most abundant alkaloid in green tea, being its amount in fresh leaves around 2–5% mass of the dry weight (Perva-Uzunalic et al., 2006; Park et al., 2007a). The effects of caffeine as stimulant of the central nervous system are well known. Some adverse effects are derived from its consumption, including sleep deprivation, tachycardia, abortion and miscarriages. Similarly to caffeine, theophylline and theobromine stimulates the central nervous system, but its amount in green tea is lower than 0.5% mass of the dry weight (Engelhardt, 2010; Zhao et al., 2011).

Regarding phenolic compounds, green tea contains large amounts of these compounds (up to 30% mass of tea solids) and catechins are the major phenolic constituents (Wei et al., 2011; Chen et al., 2006; Engelhardt, 2010). Catechins are flavonoids (flavan-3-ols) which are composed primarily of epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG). Catechins contribute to the taste of the tea and important pharmacological properties have been assigned to their consumption, such as antioxidant, anticancer, anti-inflammatory, antibiotic and antiviral effects (Rusak et al., 2008; Härdtner et al., 2012; Singh et al., 2010; Osterburg et al., 2009; Song et al., 2005; Yang et al., 2011). Thus, a selective extraction of caffeine is desirable in the manufacturing of healthy caffeine-free green tea.

The current commercially available methods for decaffeinating green tea leaves have been solvent based extraction, using chlorinated solvents like chloroform or methylene chloride, but these solvents present high toxicity. Ethyl acetate, other solvent commercially used for decaffeination, present much lesser toxicity than chlorinated solvents, but its use also diminish the quantity of catechins in the tea leaf (Engelhardt, 2010; Dong et al., 2011). In this regard, Perva-Uzunalic et al. (2006) studied the extraction efficiency of catechins and caffeine using other different liquid solvents, such as acetone, methanol, ethanol, acetonitrile as well as water. By using water, the author reached caffeine and catechins recoveries of 56–89% and 61–84%, respectively, at temperatures from 343 to 368 K and 2 h of extraction time.

Supercritical fluid extraction (SFE) has become a commercial alternative to toxic solvents. Several works about the use of supercritical CO_2 for decaffeination of tea have been reported, in which a prior treatment with water or ethanol takes place. For example, Park et al. (2007a) studied the decaffeination of green tea using CO_2 and ethanol as cosolvent at 300 bar and 343 K, attaining a caffeine recovery around 80–96% and a recovery of EGCG (the major catechin present in green tea) in the range of 46–74% (caffeine/EGCG recovery ratio ≈ 1.3–1.7). In another contribution (Park et al., 2007b), the authors employed the same extraction conditions but water as cosolvent, and in this case the caffeine/EGCG recovery ratio was lower (around 1.1). Huang et al. (2007) tested pressures from 200 to 300 bar and temperatures of 313–333 K using also water as cosolvent. Practically complete decaffeination was attained (95.6% of caffeine was removed from the green tea leaves) and the caffeine/catechins recovery ratio was 4.8. Nevertheless, it should be taken into account the small content of caffeine (9.92 mg/g) contained in the green tea utilized in their experiments. Kim et al. (2008) achieved a recovery ratio of 2.6 at 400 bar, 313 K and 7% of water as cosolvent, but only 54% of caffeine extraction yield could be reached. In conclusion, effective decaffeination can be achieved using SFE, but catechins are also significantly co-extracted, reducing the value of green tea as a functional healthy drink.

Ethyl lactate(ethyl 2-hydroxypropanoate) is an agrochemical and economically viable alternative to traditional liquid solvents, and it is fully biodegradable, non-corrosive, non-carcinogenic and non-ozone depleting. Ethyl lactate is recognized GRAS (generally recognized as safe) and due to its low toxicity was approved by the U.S. Food and Drug Administration (FDA) as pharmaceutical and food additive. These characteristics have increased the attention to the use of ethyl lactate as a green solvent for the food industry. Several reported potential applications are related with the extraction of carotenoids from different plant matrix (Ishida and Chapman, 2009; Strati and Oreopoulou, 2011), the extraction of γ-linolenic acid from Spirulina (Golmakani et al., 2012) and with the fractionation of edible oil compounds (squalene and tocopherol) (Hernández et al., 2011; Vicente et al., 2011).

The authors have studied recently (Villanueva Bermejo et al., 2013) the pressurized liquid extraction (PLE) of caffeine from vegetal materials, e.g. green coffee beans and green tea leaves, and demonstrated that ethyl lactate is a suitable solvent for decaffeination. PLE was selected as extractive method due to its faster extraction, higher yields and reduction of amount of solvent required in comparison with conventional solid–liquid extraction. Particularly, the removal of caffeine from green tea using ethyl lactate PLE was studied in the temperature range of 373–473 K, but no analysis regarding the co-extraction of catechins was informed.

In this work, the analysis of both caffeine and catechins PLE from green tea leaves using ethyl lactate, water and ethyl lactate + water mixtures is reported. Ethyl lactate + water mixtures were employed because it was observed (ambient temperature and pressure) that the solubility of caffeine in ethyl lactate increases in the presence of water. In fact, the solubility of caffeine in ethyl lactate + water mixtures shows a maximum for mixtures containing around 40% mass of water (data measured in this work). This solubility behaviour leads to presume that decaffeination can be improved using ethyl lactate + water mixtures. Yet, since preservation of catechins in the vegetal material is desirable, the recovery of both caffeine and monomeric catechins in the different extracts were determined, and the caffeine/catechins selectivity of the different solvents employed was calculated and compared.

2. Material and methods

2.1. *Samples and reagents*

"Gunpowder" green tea (*C. sinensis*) leaves were acquired in a Spanish market. The green tea leaves were ground in a cooled knife mill using liquid nitrogen (particle size smaller than 250 μm).

Ethyl lactate (≥98% purity) was obtained from Sigma-Aldrich (St. Louis, MO, USA). Acetonitrile (HPLC grade) was obtained from Lab-Scan analytical sciences (Gliwice, Polonia). Formic acid (≥98% purity) was obtained from Merck (Darmstadt, Alemania). Sea sand was obtained from Panreac Química S.A.U. (Barcelona, España).

Standards: (+)-catechin (≥99% purity), (−)-epigallocatechin (≥95% purity), (−)-epicatechin gallate (≥97.5% purity) and (−)-epigallocatechin gallate (≥98%) were purchased from Extrasynthèse (Genay, Lyon, Francia). (−)-Epicatechin (≥90% purity), caffeine (≥99% purity), theophylline (≥99% purity) and theobromine (≥99% purity) were purchased from Sigma-Aldrich.

The total content of caffeine and catechins in the vegetal material was measured. For this purpose, 200 mg of green tea leaves were extracted at 343 K with 20 mL of an aqueous ethanol solution (30% v/v) (Park et al., 2007a) in a Stuart Orbital S150 shaker apparatus (Bibby Scientific Limited Stone, UK) during 4 h. Then, the solvent was renewed and successive extraction cycles of 4 h were accomplished. The amount of caffeine recovered after two successive cycles were, respectively, 26.7 mg/g (first cycle) and 0.3 mg/g (second cycle). Regarding catechins, the corresponding yields were 84.9 and 0.1 mg/g. Thus, the content of caffeine and catechins in the vegetal material were determined to be, respectively, 27 mg and 85 mg per g of green tea leaves.

2.2. Solubility measurements

The solubility of caffeine in the liquid ethyl lactate + water solvent were measured at 298 K and atmospheric pressure as a function of the ethyl lactate/water ratio. For all solutions studied, the liquid solvent and caffeine in excess were placed into glass vessels (10 mL) with a magnetic stir bar. The vessels were put inside a water bath heated by a magnetic hotplate stirrer (RCT classic IKAMAG® safety control. IKA Works GmbH & Co. KG, Staufen, Germany) which was used to agitate the samples during 24 h under fixed temperature, controlled by an electronic contact thermometer with probe (VWR VT-5 VWR International, LLC West Chester, Pennsylvania, USA) with an accuracy of 0.2 K. After reaching the equilibrium, stirring was stopped and the vessels were left still for more than 48 h to allow a complete phase separation. Samples of clear saturated liquid solution (100 μL) were taken using a micropipette, filtered through a 0.20 μm filters and placed into glass vials. Samples were appropriately diluted for HPLC analysis.

2.3. Pressurized liquid extractions

Extractions were carried out in an Accelerated Solvent Extraction system ASE 350 from Dionex Corporation (Sunnyvale, CA, USA) equipped with a solvent controller unit. A detailed explanation of the equipment and experimental procedure is given elsewhere (Villanueva Bermejo et al., 2013).

Experiments were carried out at 323, 373, 423 and 473 K when ethyl lactate (EL) or water (W) were utilized. Moreover, two different temperatures (373 and 398 K) were selected when using 0:100%, 25:75%, 50:50%, 75:25% and 100:0% EL:W mixtures. Around 1 g of solid sample and 1 g of sea sand (used as dispersant) were employed in each experimental assay. Several preliminary tests were carried out in order to select the extraction time that was fixed at 20 min. All extracts produced were stored under refrigeration until analysis.

2.4. Identification and quantification of caffeine and catechins

Analysis were performed with an Accela (Thermo Electron Corporation, San Jose, CA) equipped with an ACE 3 C18-AR column (150 × 4.6 mm, 3 μm particle size) (Advanced Chromatography Technologies, Aberdeen, UK) equipped with a DAD detector and triple quadrupole mass spectrometer (TSQ-Quantum, Thermo Electron Corporation, San Jose, CA) with an ESI (Electrospray Ionization) interface. Based on the method of Pelillo et al. (2002), the composition of the mobile phase was (A) 0.5% (v/v) formic acid in water (B) 0.3% (v/v) formic acid in acetonitrile. The column temperature was maintained at 308 K, with a flow rate of 0.6 mL/min. The mobile phase gradient employed was as follows: initial 100% A, 30 min 0% A, 32 min 100% A, 37 min 100% A. Spray voltage and sheath gas pressure was set in 5000 and 35 psi, respectively, and the capillary temperature was 623 K. Mass analyzer was set simultaneously in full scan and SRM (Single Reaction Monitoring) modes. In this case, SRM experiments were done using 1 precursor ion and 1 daughter ion, operating in positive mode. Table 1 shows the SRM transitions selected automatically among the most abundant ions and the corresponding collision energies. The amount of target compounds in the different samples was calculated by triplicate from a calibration curve of standards.

Table 1 – Single Reaction Monitoring (SRM) transitions and the corresponding collision energies selected for the analysis of the main catechins and alkaloids of green tea.

Compound	Precursor ion	Daughter ion	Collision energy (V)
(+)-Catechin	290.971	138.987	20
Epicatechin	290.971	138.987	20
Epigallocatechin	306.932	138.989	15
Epicatechin gallate	443.029	123.034	32
Epigallocatechin gallate	459.035	138.999	23
Caffeine	195.066	138.055	19
Theophylline	181.068	124.062	18
Theobromine	181.147	163.006	9

3. Results and discussion

3.1. Solubility of caffeine in ethyl lactate + water mixtures

Table 2 shows the solubility of caffeine in ethyl lactate + water mixtures measured in this work at 298 K and atmospheric pressure. The table includes the values obtained for the pure solvents, namely ethyl lactate and water. Previous data available in the literature about the solubility of caffeine in water corresponds to values of 2.2% mass at 298 K (Bustamante et al., 2002). On the other side, the solubility of caffeine in ethyl lactate was previously reported by the authors (Manic et al., 2012) resulting in 3.2% mass at 303 K. Thus, previous data from the literature satisfactory agree with the solubilities measured in this work (2.0 and 3.0% mass at 298 K for, respectively, water and ethyl lactate).

Table 2 – Solubility of caffeine in ethyl lactate (EL) + water (W) mixtures at atmospheric pressure and 298 K.

Water content in the EL + W solvent (% mass)	Solubility (% mass of caffeine)
0	2.97 ± 0.08
9.6	5.94 ± 0.07
19.3	7.80 ± 0.07
39.0	8.04 ± 0.08
49.1	7.87 ± 0.08
59.1	6.86 ± 0.07
69.0	5.99 ± 0.09
79.2	4.56 ± 0.07
89.6	3.15 ± 0.05
100	2.00 ± 0.08

The values given in Table 2 correspond to the average value ($\bar{x}$) of experiments carried out by duplicate. Standard deviations (SD) were calculated according to the following equation:

$$SD = \sqrt{\frac{1}{2}\left[(x_1 - \bar{x})^2 + (x_2 - \bar{x})^2\right]} \quad (1)$$

being x_1 and x_2 the corresponding values obtained in each of the duplicate experiments. The average relative deviation (ARD)

$$ARD = \frac{1}{N}\sum \frac{SD_i}{\bar{x}_i} \quad (2)$$

was 1.7% for the N experiments.

The solubility of caffeine in the mixed solvent ethyl lactate + water is considerably higher than the corresponding solubility in pure ethyl lactate or water, as can be clearly observed in Table 2. That is, the addition of water to the ethyl lactate solvent can substantially increase caffeine solubility, attaining a maximum around 8% mass for a mixed solvent comprising 40% mass of water. This solubility value is around 2.7 fold higher than the solubility in pure ethyl lactate and 4.0 fold higher than in water.

3.2. *Pressurized liquid extraction of green tea leaves*

3.2.1. *PLE extraction using ethyl lactate or water*

Preliminary studies were carried out in order to set the extension of the static extraction experiments. Sequential steps of 10 min were carried out at the highest temperature explored (473 K) using ethyl lactate or water. Around 15–20% of the yield obtained during the first step was recovered in the second step, while yields lower than 2% were attained in a third step. Thus, extraction time was set to 20 min in order to proceed with the study of (i) the effect of temperature and (ii) the use of ethyl lactate + water mixtures on the extraction of caffeine from green tea leaves. In this study, considering the co-extraction of the valuable bioactive catechins, the concentrations of EC, ECG, EGC, EGCG and (+)-catechin in the extracts were also determined. Additionally, two other alkaloids present in green tea leaves, theophylline and theobromine were identified and quantified.

First, the PLE of green tea leaves using pure ethyl lactate or water was accomplished in the temperature range of 323–473 K. The corresponding results are given in Table 3. The ARD calculated according to Eq. (2) were 5.5% and 7.6%, respectively, for the extraction yield and the concentration (% mass) of the target compounds in the extracts.

As can be observed in Table 3, higher extraction yields were obtained with water, but the increase of yield with temperature is particularly noteworthy when ethyl lactate is employed as extractive solvent (see Fig. 1).

Concerning the analysis of bioactive compounds, caffeine was the main alkaloid extracted and the concentration of theophylline and theobromine were lower than 0.2% mass.

In the case of ethyl lactate, the increase of temperature increases the concentration of both, caffeine and catechins, attaining a maximum around 373–423 K and then decreasing (see Table 3). When water is employed as solvent, the concentration of caffeine in the extracts is almost constant while catechins concentrations decrease with increasing temperature. Fig. 2 compare the recovery of caffeine (Fig. 2a) and catechins (Fig. 2b) for each solvent employed. The recovery of caffeine increases with temperature and attain values higher than 75% at temperatures higher than 423 K. In this regard, almost complete decaffeination was achieved using ethyl lactate at 473 K (92% of caffeine was removed). On the other side, the recovery of catechins increase up to 423 K and then decrease, denoting the possibility of thermal degradation. This effect is especially pronounced when water is used unlike ethyl lactate. Thus, for both solvents, extraction temperature lower than 423 K is recommended in order to avoid thermal degradation of catechins. Furthermore, ethyl lactate can extract similar amounts of caffeine than water at temperatures around 373–423 K, while considerable lower amounts of catechins are extracted from the green tea leaves (see Fig. 2).

Table 3 – Extraction yield (Y = mass extracted/mass of raw material × 100), concentration of key bioactives* (% mass) and caffeine/catechins solvent selectivity (Eq. (5)) corresponding to the PLE of green tea leaves. Extraction time: 20 min.

Solvent	Temperature (K)			
	323	373	423	473
Ethyl lactate				
Yield (Y)	3.9	10.8	16.0	20.8
Caffeine (% mass)	7.4	12.5	12.8	11.9
Catechins (% mass)	16.5	20.5	19.3	11.7
Selectivity	1.5	2.8	5.5	27.4
Water				
Yield (Y)	20.6	30.0	40.4	43.8
Caffeine (% mass)	5.5	4.8	5.1	4.7
Catechins (% mass)	16.7	12.2	9.4	2.7
Selectivity	1.1	1.5	4.0	19.9

* Catechins: (+)-catechin, (−)-EC, (−)-EGC, (−)-ECG, (−)-EGCG. Theobromine concentrations lower than 0.2% mass. Theophylline: below quantification level.

The selectivity (S) of each solvent towards the extraction of the two types of solutes, caffeine (1) and catechins (2), was calculated according to

$$S = \frac{k_1}{k_2} \quad (3)$$

where k_1 and k_2 are, respectively, the distribution coefficient of caffeine and catechins, which were calculated considering

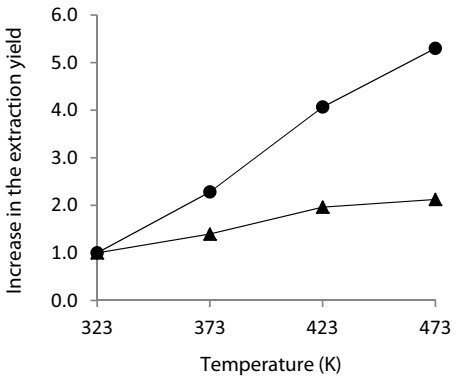

Fig. 1 – Increase with temperature of the extraction yield ($Y_{(T)}/Y_{(323\ K)}$) in the PLE of green tea leaves using (●) ethyl lactate or (▲) water.

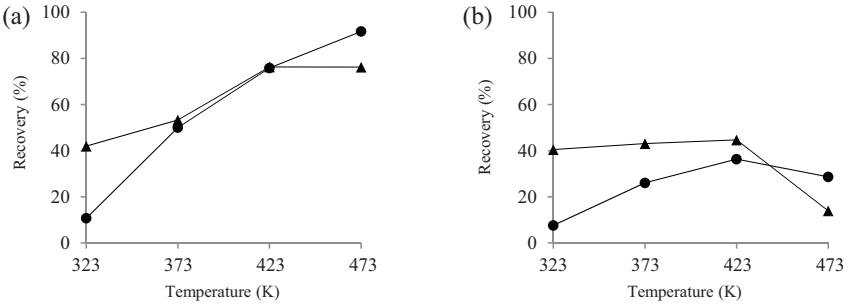

Fig. 2 – Recovery (mass extracted/initial mass of the compound in vegetal matter × 100) of (a) caffeine and (b) catechins as a function of temperature in the PLE of green tea leaves using (●) ethyl lactate or (▲) water. Initial mass of caffeine and catechins determined in the raw material: 27 mg/g and 85 mg/g of green tea, respectively.

the concentration (% mass) of the solute in the extract (y_i^{ext}) and in the residual vegetal material (x_i^{sol}), that is

$$k_i = \frac{y_i^{ext}}{x_i^{sol}} \quad (4)$$

The y_i^{ext} values for caffeine and catechins are given in Table 3, and the x_i^{sol} concentrations in the extracted vegetal material were calculated according to

$$x_i^{sol} = \frac{m_i^{sol}}{(1 - Y/100)} \quad (5)$$

being m_i^{sol} the non-extracted solute per unit mass of raw material employed and Y the corresponding extraction yield (see Table 3). The non-extracted solute was calculated as the difference between the initial solute content in the raw material (27 mg/g of caffeine and 85 mg/g of catechins, respectively) and the mass of solute extracted ($y_i^{ext} \cdot Y$).

The calculated selectivity of water and ethyl lactate towards the extraction of caffeine and catechins is reported in Table 3. The selectivity of ethyl lactate is higher than the selectivity of water over the whole range of studied temperatures. The large increase of selectivity values observed at 473 K for both solvents (particularly for water) should not be taken into account due to the fact that although at this temperature almost the whole caffeine was removed, thermal degradation of catechins was produced, as noted above. In this respect, the selectivity calculated at 423 K may be also overestimated since the thermal stability of catechins at this temperature is not confirmed.

3.2.2. *PLE extraction using ethyl lactate + water mixtures*

Considering the solubility measurements and the results obtained from the PLE-temperature dependence study, the extraction of green tea leaves using ethyl lactate (EL) + water (W) mixtures (instead of pure solvents) was accomplished. Extraction temperatures were selected in order to avoid catechins thermal degradation (373 and 398 K) and different EL:W ratios (0:100%, 25:75%, 50:50%, 75:25% and 100:0%) were employed.

Table 4 shows the extraction yield, concentration of caffeine and catechins and solvent selectivity for both temperatures studied. As can be observed, larger yields were obtained with the mixed solvent in comparison with the pure solvents. The addition of water to ethyl lactate significantly increased the PLE yield at both temperatures investigated. Nevertheless, regarding the concentration (mass %) of the target compounds in the extracts, the addition of water results in a decrease in the concentration of both caffeine and catechins, with values closer to those obtained using pure water. Consequently, the caffeine/catechins selectivity was not improved with respect to the values obtained with pure ethyl lactate. The highest selectivity attained was 2.8, at 373 K and with 50:50% ethyl lactate + water, which is the same value obtained using pure ethyl lactate at the same temperature. Nevertheless, it should be pointed out that with the 50:50% solvent, better decaffeination (higher caffeine recovery) could be achieved.

The recoveries of caffeine and catechins are depicted in Fig. 3 as a function of the EL:W ratio and considering the two different temperatures tested. In general, higher recoveries were attained for the mixed solvent in comparison with the pure solvents. However, as mentioned before, the caffeine/catechins selectivity for all EL:W ratios employed were not higher than the selectivity obtained with pure ethyl lactate.

Table 5 shows the concentration of each catechin in the extracts (% mass normalized). Similar concentrations were obtained for the identified catechins (EC, (+)-cat, ECG, EGCG, EGC) despite the solvent employed (ethyl lactate, water or mixtures ethyl lactate + water). For all extracts obtained, EGCG was the most abundant catechin obtained in the extracts (around 50–60% of total catechins) under all extraction conditions, followed by EGC and ECG (around 20% each).

Table 4 – Extraction yield (Y = mass extracted/mass of raw material × 100), concentration of key bioactives* (% mass) and caffeine/catechins solvent selectivity (Eq. (5)) corresponding to the PLE of green tea leaves using as solvent different ethyl lactate (EL) + water (W) mixtures. Extraction time: 20 min.

Temperature	EL:W ratio				
	100:0	75:25	50:50	25:75	0:100
373 K					
Yield (Y)	10.8	37.2	39.6	42.4	30.0
Caffeine (% mass)	12.5	5.8	5.1	4.3	4.8
Catechins (% mass)	20.5	13.5	11.0	10.2	12.2
Selectivity	2.8	2.7	2.8	2.0	1.5
398 K					
Yield (Y)	19.9	41.0	50.3	65.2	37.3
Caffeine (% mass)	9.0	5.1	4.5	3.0	3.1
Catechins (% mass)	13.6	12.6	11.3	7.9	8.7
Selectivity	4.2	2.2	2.6	1.7	1.2

* Catechins: (+)-catechin, (−)-EC, (−)-EGC, (−)-ECG, (−)-EGCG. Theobromine concentrations lower than 0.2% mass. Theophylline: below quantification level.

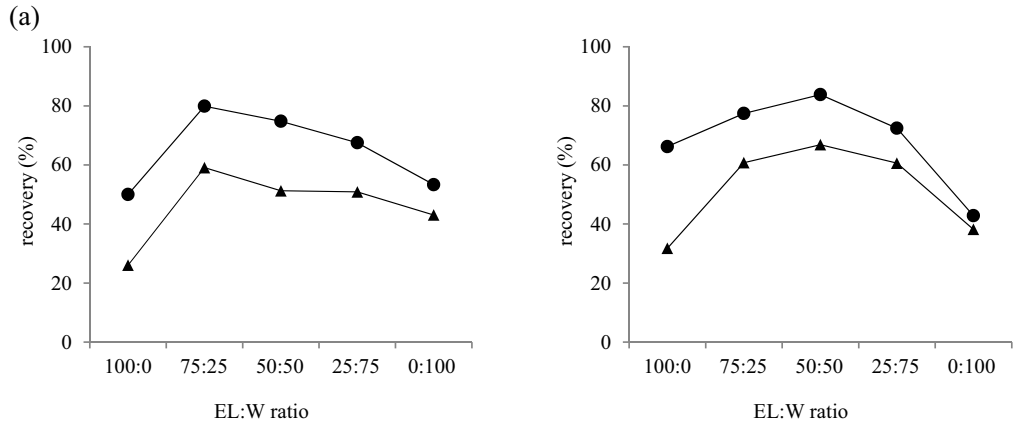

Fig. 3 – Recovery (mass extracted/initial mass of the compound in vegetal matter × 100) of (●) caffeine and (▲) catechins as a function of the EL:W ratio for the PLE of green tea leaves at (a) 373 K and (b) 398 K.

Table 5 – Concentration of the different catechins (% mass normalized) corresponding to the PLE of green tea leaves using as solvent (a) ethyl lactate, (b) water and (c) ethyl lactate (EL) + water (W) mixtures. Extraction time: 20 min..

Solvent		Compound					
(a) Ethyl lactate				T (K)			
				323	373	423	473
		(+)-cat.		0.8	1.9	1.9	2.1
		EC		9.0	9.5	11.0	9.4
		EGC		17.6	22.4	16.0	17.4
		ECG		20.3	20.5	25.9	28.1
		EGCG		52.3	45.8	45.2	43.0
(b) Water				T (K)			
				323	373	423	473
		(+)-cat.		0.7	1.5	1.7	2.0
		EC		10.6	8.4	7.7	2.3
		EGC		26.9	23.4	20.7	23.3
		ECG		13.4	12.9	23.8	22.9
		EGCG		48.3	53.8	46.1	49.5
(c) EL + W				EL:W ratio			
	T (K)		100:0	75:25	50:50	25:75	0:100
	373						
		(+)-cat.		1.7	2.3	2.1	
		EC		6.7	8.0	7.0	
		EGC		22.9	16.8	20.4	
		ECG		15.5	20.2	17.3	
		EGCG		53.2	52.8	53.2	
	398						
		(+)-cat.	2.2	2.0	2.1	2.8	2.1
		EC	8.2	6.8	6.4	6.4	5.9
		EGC	25.9	22.6	20.3	15.2	17.7
		ECG	16.5	16.4	16.5	15.5	14.3
		EGCG	47.3	52.3	54.7	60.0	60.0

4. Conclusions

PLE using pure ethyl lactate is a potential suitable alternative to remove caffeine from natural matter, and in the case of green tea leaves, the co-extraction of catechins is minimized in comparison with the extraction using other liquid solvents or supercritical CO_2. For example, at 373 K the caffeine/catechins selectivity was 2.8 (no thermal degradation of catechins was observed at this temperature) and the recovery of caffeine and catechins were, respectively, 53% and 26%. Then, the caffeine/catechin recovery ratio was 2.0, higher than those obtained in this work using water or those reported by Perva-Uzunalic et al. (2006) using other solvents. In comparison with $SCCO_2$ extraction, previous work (Park et al., 2007a, 2007b) reported caffeine/catechins recovery ratios in the range 1.3–1.7 with suitable decaffeination (80–96% caffeine removal). At higher temperatures (423–473 K), ethyl lactate PLE can remove 76–92% of caffeine, but the higher caffeine/catechins selectivity obtained cannot be confirmed due to possible thermal degradation of catechins.

On the other side, with the ethyl lactate + water mixtures, higher caffeine recoveries were obtained but the recoveries of

catechins were increased even more and thus, selectivity factors were not improved with respect to ethyl lactate, and were similar to those obtained with pure water. Nevertheless, it should be taken into account that these recoveries are 1.4–1.8 (EL:W ratios around 75:25%) higher than those obtained using water, and can be considered a good alternative to recover bioactives from green tea leaves.

The results presented in this work show the effective use of ethyl lactate, water and ethyl lactate + water mixtures to remove caffeine form green tea leaves. The co-extraction of catechins was also examined since these substances are the most important bioactives of green tea. The best caffeine/catechins selectivity in the decaffeination process was obtained using pure ethyl lactate.

In addition to caffeine and catechins, other bioactive compounds (proanthocyanidins, quercetin and kaempferol glycosides, theanine) could be present in the extracts produced using the solvents studied in this work. Furthermore, it should be considered also the co-extraction of lipids, favoured with 100% ethyl lactate, and carbohydrates, highly soluble in water, which also might be extracted. This work establishes the basis of further studies, including the analysis of the co-extraction of these compounds and their impact on the organoleptic quality of the infusion that can be produced after decaffeination.

Acknowledgements

D. Villanueva acknowledges the predoctoral contract (JAE) given by the Consejo Superior de Investigaciones Científicas (CSIC) of Spain. This work was financed thanks to AGL2011-29857-C03-01 (Ministerio de Economía y Competitividad) and ALIBIRD, S2013/ABI-2728 (Comunidad de Madrid) projects.

References

Bustamante, P., Navarro, J., Romero, S., Escalera, B., 2002. Thermodynamic origin of the solubility profile of drugs showing one or two maxima against the polarity of aqueous and nonaqueous mixtures: niflumic acid and caffeine. J. Pharm. Sci. 91, 874–883.

Chen, Q., Zhao, J., Huang, X., Zhang, H., Liu, M., 2006. Simultaneous determination of total polyphenols and caffeine contents of green tea by near-infrared reflectance spectroscopy. Microchem. J. 83, 42–47.

Dong, J., Ye, J., Lu, J., Zheng, X., Liang, Y., 2011. Isolation of antioxidant catechins from green tea and its decaffeination. Food Bioprod. Process. 89, 62–66.

Engelhardt, U.H., 2010. Comprehensive natural products II: Chemistry and biology. In: Mander, L., Liu, H.B. (Eds.), Chemistry of Tea, vol. 3. Elsevier Applied Science Publishers Ltd, London, pp. 999–1032.

Golmakani, M., Mendiola, J.A., Rezaeic, K., Ibáñez, E., 2012. Expanded ethanol with CO_2 and pressurized ethyl lactate to obtain fractions enriched in (-linolenic acid from *Arthrospira platensis* (Spirulina). J. Supercrit. Fluids 62, 109–115.

Härdtner, C., Multhoff, G., Falk, W., Radons, J., 2012. (−)-Epigallocatechin-3-gallate, a green tea-derived catechin, synergizes with celecoxib to inhibit IL-1-induced tumorigenic mediators by human pancreatic adenocarcinoma cells Colo357. Eur. J. Pharmacol. 684, 36–43.

Hernández, E.J., Luna, P., Stateva, R.P., Najdanovic-Visak, V., Reglero, G., Fornari, T., 2011. Liquid–liquid phase transition of mixtures comprising squalene, olive oil, and ethyl lactate: application to recover squalene from oil deodorizer distillates. J. Chem. Eng. Data 56, 2148–2152.

Huang, K., Wu, J., Chiu, Y., Lai, C., Chang, C., 2007. Designed polar cosolvent-modified supercritical CO_2 removing caffeine from and retaining catechins in green tea powder using response surface methodology. J. Agric. Food Chem. 55, 9014–9020.

Ishida, B.K., Chapman, M.H., 2009. Carotenoid extraction from plants using a novel, environmentally friendly solvent. J. Agric. Food Chem. 57, 1051–1059.

Kim, W., Kim, J., Kim, J., Oh, S., Lee, Y., 2008. Selective caffeine removal from green tea using supercritical carbon dioxide extraction. J. Food Eng. 89, 303–309.

Manic, M.S., Villanueva, D., Fornari, T., Queimada, A.J., Macedo, E.A., Najdanovic-Visak, V., 2012. Solubilities of high-value compounds in ethyl lactate: measurements and modelling. J. Chem. Thermodyn. 48, 93–100.

Osterburg, A., Gardner, J., Hyon, S.H., Neely, A., Babcock, G., 2009. Highly antibiotic-resistant *Acinetobacter baumannii* clinical isolates are killed by the green tea polyphenol (–)-epigallocatechin-3-gallate (EGCG). Microbiol. Infect. 15, 341–346.

Park, H.S., Choi, H.K., Lee, S.J., Park, K.W., Choi, S.G., Kim, K.H., 2007a. Effect of mass transfer on the removal of caffeine from green tea by supercritical carbon dioxide. J. Supercrit. Fluids 42, 205–212.

bPark, H.S., Lee, H.J., Shin, M.H., Lee, K.W., Lee, H., Kim, Y.S., Kim, K.O., Kim, K.H., 2007b. Effects of cosolvents on the decaffeination of green tea by supercritical carbon dioxide. Food Chem. 105, 1011–1017.

Pelillo, M., Biguzzi, B., Bendini, A., GallinaToschi, T., Vanzini, M., Lercker, G., 2002. Preliminary investigation into development of HPLC with UV and MS-electrospray detection for the analysis of tea catechins. Food Chem. 78, 369–374.

Perva-Uzunalic, A., Skerget, M., Knez, Z., Weinreich, B., Otto, F., Gruner, S., 2006. Extraction of active ingredients from green tea (*Camellia sinensis*): extraction efficiency of major catechins and caffeine. Food Chem. 96, 597–605.

Rusak, G., Komes, D., Likic, S., Horzic, D., Kovac, M., 2008. Phenolic content and antioxidative capacity of green and white tea extracts depending on extraction conditions and the solvent used. Food Chem. 110, 852–858.

Singh, R., Akhtar, N., Haqqi, T.M., 2010. Green tea polyphenol epigallocatechin-3-gallate: inflammation and arthritis. Life Sci. 86, 907–918.

Song, J., Lee, K., Seong, B., 2005. Antiviral effect of catechins in green tea on influenza virus. Antivir. Res. 68, 66–74.

Strati, I.F., Oreopoulou, V., 2011. Effect of extraction parameters on the carotenoid recovery from tomato waste. Int. J. Food Sci. Technol. 46, 23–29.

Vicente, G., Paiva, A., Fornari, T., Najdanovic-Visak, V., 2011. Liquid–liquid equilibria for separation of tocopherol from olive oil using ethyl lactate. Chem. Eng. J. 172, 879–884.

Villanueva Bermejo, D., Luna, P., Manic, M., Najdanovic-Visak, V., Reglero, G., Fornari, T., 2013. Extraction of caffeine from natural matter using a bio-renewable agrochemical solvent. Food Bioprod. Process. 91, 303–309.

Wei, K., Wang, L., Zhou, J., He, W., Zeng, J., Jiang, Y., Cheng, H., 2011. Catechin contents in tea (*Camellia sinensis*) as affected by cultivar and environment and their relation to chlorophyll contents. Food Chem. 125, 44–48.

Yang, C.S., Wang, H., Li, G.X., Yang, Z., Guan, F., Jin, H., 2011. Cancer prevention by tea: evidence from laboratory studies. Pharmacol. Res. 64, 113–122.

Zhao, Y., Chen, P., Lin, L., Harnly, J.M., Yu, L., Li, Z., 2011. Tentative identification, quantitation, and principal component analysis of green pu-erh, green, and white teas using UPLC/DAD/MS. Food Chem. 126, 1269–1277.

3.2.2. Extracción supercrítica de cafeína de hojas de té verde utilizando lactato de etilo como modificador

J. of Supercritical Fluids xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

The Journal of Supercritical Fluids

journal homepage: www.elsevier.com/locate/supflu

Effect of cosolvents (ethyl lactate, ethyl acetate and ethanol) on the supercritical CO_2 extraction of caffeine from green tea

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ARTICLE INFO

Article history:
Received 16 May 2015
Received in revised form 5 July 2015
Accepted 6 July 2015
Available online xxx

Keywords:
Green tea
Caffeine
Cosolvent
Ethyl lactate
Supercritical fluid extraction

ABSTRACT

This paper reports experimental data to analyze the effect of different green cosolvents, namely ethyl lactate, ethyl acetate and ethanol, on the supercritical carbon dioxide ($SCCO_2$) extraction of caffeine from green tea leaves.

The experiments were carried out in a pilot-scale plant using two different approaches: a static procedure in which the cosolvent was introduced in the extraction cell soaking the vegetal material and then $SCCO_2$ was pumped, and a dynamic assay in which the cosolvent was pumped and mixed with $SCCO_2$ before introduction into the extraction cell. The overall caffeine recovery from plant matrix was determined for all experiments at the same extraction conditions (30 MPa and 343 K). Additionally, the overall extraction curves of the static experiments were determined at the same process conditions, and the mass transfer model of Sovová was utilized to adjust the kinetic data and to determine the mass transfer coefficients.

The highest caffeine yield was obtained with ethyl lactate in both static and dynamic extractions (13.0 and 14.2 mg/g of tea, respectively), followed by ethanol (10.8 mg/g with the static method and 8.8 mg/g with the dynamic method). The yield obtained with ethyl acetate in both extraction approaches was lower than 7 mg/g. These data reinforce previous results obtained by the authors regarding the competence of ethyl lactate in the extraction of caffeine from natural materials.

1. Introduction

Green tea (*Camellia sinensis*) is one of the most popular beverages in the world [1] and contains around 2–5% mass of caffeine (1,3,7-trimethylxanthine) in the dry leaves [2]. Some adverse effects derived from the excessive consumption of caffeine are well-known [3–5] and thus, the removal of caffeine is desired by several consumers.

Extraction using organic solvents is currently the most extended commercial method for decaffeinating green tea leaves. Despite the high selectivity towards caffeine, the use of solvents like chloroform or methylene chloride has progressively decline due to their high toxicity [6]. Another commercial solvent utilized is ethyl acetate which presents much lesser toxicity than chlorinated solvents.

Supercritical fluid extraction (SFE) with CO_2 is a viable alternative to organic solvents, offering many advantages such as non-toxicity, solvent-free products and high selectivity in caffeine removal. Several works about the use of supercritical CO_2 for decaffeination of different vegetal matrix, such as coffee beans [7], cocoa butter [8], guarana seeds [9] and mate tea leaves [10], have been reported. In fact, the extraction of caffeine with supercritical CO_2 is one of the most well-known commercial examples of SFE processes [11]. Many patents have been published about decaffeination of tea and coffee where extraction of caffeine is carried out by means of different layouts [12–15]. Moreover, in different countries, several large-scale plants have been designed for processing coffee, tea, and hops, and for spices and flavor extraction [16].

Regarding the extraction of caffeine from green tea leaves, in most cases, green tea was soaked previously with a liquid solvent, namely ethanol or water. For example, Park et al. [17,18] studied the decaffeination of green tea using CO_2 and ethanol as cosolvent at 30 MPa and 343 K, attaining a recovery of caffeine around 92–96%. In another contribution [19], the authors employed the same extraction conditions with pure CO_2 and water as cosolvent and caffeine recoveries were lower. Similarly, Kim et al. [20] attained a 59% of caffeine recovery at 40 MPa, 323 K and using 7% of water as cosolvent. Chang et al. [21] tested water, ethanol and

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http://dx.doi.org/10.1016/j.supflu.2015.07.008
0896-8446/

different water/ethanol ratios at 31 MPa and 333 K, obtaining the best results with 70% aqueous ethanol.

On the other side, Sun et al. [22] studied the supercritical extraction of caffeine also using ethanol and water, but the cosolvent was pumped and mixed with $SCCO_2$ in continuous flow (the vegetal material was not soaked previously). At 30 MPa and 333 K the recovery of caffeine using ethanol, 50% aqueous ethanol and water was, respectively, 67.4%, 92.8%, 78.0%.

Huang et al. [23] combined soaking and continuous CO_2+cosolvent pumping. Complete decaffeination was attained at 30 MPa and 353 K, but it should be taken into account the small content of caffeine (9.92 mg/g) contained in the green tea utilized in their experiments.

Thus, to the best of our knowledge, only water and ethanol have been studied as cosolvents in the supercritical extraction of caffeine from green tea leaves. In this work, the kinetic behaviour of the caffeine extraction when ethyl lactate and ethyl acetate are employed as cosolvents is presented for the first time. For comparison, also caffeine extraction using ethanol was analyzed and reported. Furthermore, the two most common approaches, i.e., previous soaking of the vegetal material and continuous CO_2 and cosolvent pumping, were investigated.

2. Material and methods

2.1. Samples and reagents

"Gunpowder" green tea (*C. sinensis*) leaves were acquired in a Spanish market. The green tea leaves were ground (particle size smaller than 500 μm) in a cooled knife mill (Grindomix GM 200. Retsch GmbH, Haan, Germany) using liquid nitrogen.

Ethyl lactate (≥98% purity) and caffeine standard (≥99% purity) were obtained from Sigma–Aldrich (St. Louis, MO, USA). Ethyl acetate and acetonitrile (HPLC grade) were obtained from Lab-Scan analytical sciences (Gliwice, Polonia). Formic acid (≥98% purity) was obtained from Merck (Darmstadt, Alemania). Ethanol (≥99% purity) and sea sand were purchased from Panreac Química S.A.U. (Barcelona, España). CO_2 was provided by Air Products (Allentown, PA, USA).

The total content of caffeine in the vegetal material was measured. For this purpose, 200 mg of green tea leaves were extracted at 343 K with 20 mL of an aqueous ethanol solution (30% *v/v*) [17] in a Stuart Orbital S150 shaker apparatus (Bibby Scientific Limited Stone, UK) during 4 h. Then, the solvent was renewed and successive extraction cycles of 4 h were accomplished until the extraction yield in the corresponding cycle was lower than 2% of total yield. The content of caffeine determined was 27 mg per g of green tea leaves.

2.2. Supercritical extraction method

Extractions were carried out in a pilot-scale supercritical fluid extractor (Thar Technology, Pittsburgh, PA, USA, model SF2000) comprising a 2 L cylinder extraction cell and two different separators (S1 and S2), each of 0.5 L capacity, with independent control of temperature and pressure. A detailed explanation of the experimental device can be found elsewhere [24].

The extractions were carried out using pure CO_2 and CO_2 with three different cosolvents, namely ethyl lactate (CO_2 + EL), ethanol (CO_2 + ETOH) and ethyl acetate (CO_2 + EAC).

Based on the experimental information available in the literature [17,18,25,26], the extraction pressure and temperature were selected to be 30 MPa and 343 K and were kept constant for all experimental assays. At these conditions high caffeine solubility in $SCCO_2$ is attained [25]. Furthermore, a supercritical procedure for the decaffeination of moistened green tea leaves has been early described at 293–353 K and pressure up to 30 MPa [26]. The extraction cell was loaded with 0.7 kg of green tea leaves and CO_2 flow rate was set to 9 kg/h. For the extractions using cosolvent, the cosolvent/CO_2 ratio (kg/kg) was 0.022 (around 2% mass). These extractions were conducted in two different modes: static and dynamic modes. In the first case, green tea was soaked with the cosolvent and was loaded into the extraction cell. The soaked leaves were heated up to 343 K, then CO_2 was pumped until the extraction pressure was attained. The mixture was allowed to stand during 30 min and finally, CO_2 was pumped afterward during 3.5 h (210 min). In the dynamic mode, the dry grounded leaves were loaded into the extraction cell and heated up to 343 K, then CO_2 and the cosolvent were continuously pumped and mixed in the desired ratio previous to introduction into the cell.

Decompression of the extract up to ambient pressure was accomplished in a separator at 303 K. Extraction yield was calculated as the difference between the mass of green tea leaves loaded in the extraction cell and the mass of leaves remained in the cell after the extraction. Samples were collected at different intervals of time. Samples were stored at 253 K in the dark until analysis.

Table 1
Single Reaction Monitoring (SRM) transitions and the corresponding collision energies selected for the analysis of the main catechins and caffeine of green tea.

Compound	Precursor ion	Daughter ion	Collision energy (V)
(+)−catechin	290.971	138.987	20
Epicatechin	290.971	138.987	20
Epigallocatechin	306.932	138.989	15
Epicatechin gallate	443.029	123.034	32
Epigallocatechin gallate	459.035	138.999	23
Caffeine	195.066	138.055	19

2.3. Identification and quantification of caffeine and catechins

Analysis was performed by HPLC-MS/MS with an Accela (Thermo Electron Corporation; San Jose, CA) equipped with an ACE 3C18-AR column (150 × 4.6 mm, 3 μm particle size) (Advanced Chromatography Technologies, Aberdeen, UK) equipped with a DAD detector and triple quadrupole mass spectrometer (TSQ-Quantum, Thermo Electron Corporation, San Jose, CA) with an ESI (Electrospray Ionization) interface. Based on the method of Pelillo et al. [27] the composition of the mobile phase was (A) 0.5% (*v/v*) formic acid in water (B) 0.3% (*v/v*) formic acid in acetonitrile. The column temperature was maintained at 308 K, with a flow rate of 0.6 mL/min. The mobile phase gradient employed was as follows: initial 100% A, 30 min 0% A, 32 min 100% A, 37 min 100% A. Spray voltage and sheath gas pressure was set in 5000 and 35 psi respectively and the capillary temperature was 623 K. Mass analyzer was set simultaneously in full scan and SRM (Single Reaction Monitoring) modes. In this case, SRM experiments were done using 1 precursor ion and 1 daughter ion, operating in positive mode. Table 1 shows the SRM transitions selected automatically among the most abundant ions and the corresponding collision energy. The amount of caffeine and catechins in the different samples was calculated by triplicate from a calibration curve of standard.

3. Results and discussion

Table 2 shows the total extraction yield (g of extract/g of tea) and the caffeine yield (mg caffeine extracted/g tea) obtained for both extraction approaches (static and dynamic) and for each cosolvent studied.

The dynamic and static extractions with ethyl lactate were carried out by duplicate. The mean values of the total extraction yields are reported in Table 2 and the standard deviations were,

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Table 2
Extraction yield (g of extract/g of tea), caffeine concentration (g of caffeine/g of extract), caffeine yield (mg of caffeine/g of tea) and caffeine recovery (g of caffeine extracted/g of caffeine in tea) in the static and dynamic SFE of green tea leaves using pure CO_2, CO_2 + EL, CO_2 + ETOH and CO_2 + EAC. Extraction conditions: 30 MPa and 343 K.

Extraction mode	Solvent	Extraction yield (%)	Caffeine concentration (%)	Caffeine yield (mg/g)	Caffeine recovery (%)
	Pure CO_2	3.7	15.9	5.9	21.9
Static	CO_2 + EL	9.1[a]	14.3	13.0	48.3
	CO_2 + ETOH	10.9	9.9	10.8	40.2
	CO_2 + EAC	8.1	8.1	6.6	24.6
Dynamic	CO_2 + EL	7.8[a]	18.2	14.2	52.6
	CO_2 + ETOH	8.7	10.1	8.8	32.6
	CO_2 + EAC	7.5	5.5	4.1	15.2

[a] Mean value between duplicate experiments.

respectively, 0.3 and 0.2%. Standard deviation obtained in the quantification of caffeine was lower than 0.1 mg/g.

Despite the extraction type, the effect of the cosolvent on the recovery of caffeine was as follows: ethyl lactate > ethanol > ethyl acetate. This behavior may be explained considering that hydrogen bonding would constitute one of the most important forces taking into account the chemical structure of the cosolvents and solute studied in this work [18,22,25,28]. In this respect, ethyl lactate can develop hydrogen bonding owing to its hydroxyl and carbonyl group [29] while ethanol only possesses a hydroxyl group. On the other side, ethyl acetate is a polar aprotic solvent that do not possess donor hydrogen atoms and cannot form strong hydrogen bonds. Thus, in comparison with pure CO_2 (see Table 2) caffeine recovery was higher using ethanol or ethyl lactate cosolvents, but with ethyl acetate recoveries were similar and even lower (dynamic mode).

Additionally, solubility behaviour of caffeine in the liquid solvents also supports the observed behavior. The solubility of caffeine in ethyl lactate at 323 K is 5.1% mass [30], while solubility in ethanol and ethyl acetate at the same temperature is, respectively, 2.3% and 2.2% mass [31].

The highest caffeine extraction yield was obtained with CO_2 + EL and the recovery was slightly higher in the case of the dynamic approach (13.0 mg/g and 14.2 mg/g in the static and dynamic modes, respectively). Nevertheless, in the case of ethanol and ethyl acetate cosolvents, recoveries were considerably higher in the static mode.

Taking into account the total content of caffeine in the green tea leaves (27.0 mg/g), the recovery of caffeine (mass of caffeine in the extract/mass of caffeine in green tea leaves × 100) at 343 K, 30 MPa and 210 min of extraction were 48.3% and 52.6% using CO_2 + EL in the static and dynamic modes respectively, while recoveries lower than 40% were obtained with the other cosolvents (see Tables 2 and 3). This result is in agreement with previous results reported by the authors [32] regarding the effectiveness of ethyl lactate in comparison with ethanol or ethyl acetate in the pressurized liquid extraction of caffeine from green coffee beans.

The selectivity of the cosolvents to extract caffeine was valued considering the concentration of caffeine in the extracts (see Table 2). As expected, pure CO_2 produced extracts with high content of caffeine (15.9% mass) demonstrating high selectivity to extract this alkaloid, although recovery was the lowest (21.9%). Among the cosolvents investigated, ethyl lactate produced the highest caffeine concentrations, with values similar (14.3% mass in static mode) or even higher (18.2% mass in dynamic mode) than pure CO_2 and the highest caffeine recoveries as mentioned previously.

Assessment of the co-extraction of substances other than caffeine is also important since these substances may affect the sensorial quality and/or bioactivity functions of green tea. In this respect, the content of catechins was determined by HPLC in the whole extract of the static assays, and resulted in values of 0.2, 0.8 and 6.7 mg catechins/g tea, respectively, for EA, EL and ETOH cosolvents. The caffeine/catechins selectivity can be pondered considering the ratio caffeine/catechins extracted per g of green tea leaves, which resulted 33 for EA, 16.3 for EL and 1.6 for ETOH. The cosolvent polarity is influencing the co-extraction of catechins and thus the caffeine/catechins selectivity.

Fig. 1 shows the overall extraction curve (OEC) obtained using pure CO_2 and the three cosolvents in the static-SFE. The highest extraction rates of caffeine at the early extraction stages were obtained with CO_2 + EL, while the lower extraction rates resulted with pure CO_2. The early extraction rate of caffeine were calculated on the basis of the first kinetic data collected (after 14 min of CO_2 flow) and were 0.063 mg/min in the case of pure CO_2 and 0.098 mg/min, 0.289 mg/min and 0.486 mg/min, respectively, for CO_2 + EAC, CO_2 + ETOH and CO_2 + EL. Then, ethyl lactate was the most efficient cosolvent and can produce a caffeine recovery enhancement close to 7 in comparison with pure CO_2.

The OECs of caffeine static-SFE are depicted in Fig. 1 for the case of pure CO_2 (Fig. 1a), CO_2 + EAC (Fig. 1b), CO_2 + ETOH (Fig. 1c) and CO_2 + EL (Fig. 1d). The OECs were adjusted using the model of Sovová [33] which is based on assumption that X_p mass of solute is easy accessible to the supercritical solvent (due to cell wall disruption) while the rest (X_k) remains inside cell walls. Thus, three steps are considered in the SFE process: (i) the constant rate period, where only the easily accessible solute is removed and thus, is controlled by convection in the fluid phase; (ii) the falling rate period, where both convection and internal mass transfer are important; (iii) the internal diffusion controlled rate period, where the remaining solute is only inside the cell walls. The corresponding model equations describing each extraction step are given in the Appendix A.

Furthermore, it is considered that the supercritical solvent flows axially through a cylindrical extraction bed, the solvent is solute-free at the bed inlet and particle size distribution is homogeneous throughout the extraction cell.

The solid particle density (ρ_s) is 1046 kg/m^3, bed porosity (ϵ) was determined on the basis of the corresponding apparent density (411.8 kg/m^3) and resulted $\epsilon = 0.6$, and CO_2 density (ρ) at 30 MPa and 343 K was determined from thermodynamic tables ($\rho = 788.6$ kg/m^3) [34].

Due to the lack of experimental solubility data of caffeine in the CO_2 + EL and CO_2 + EAC supercritical solvents, the solubility (Y^*) was estimated as the slope of a theoretical linear behavior of the OEC between $t = 0$ and $t = 14$ min (Table 4). For comparison, the estimated caffeine solubility in pure CO_2 was 0.029% mass, which is quite in agreement with the experimental solubility measured at 343 K and 28.1 MPa (0.023% mass) [35]. These estimated solubilities presume that, as in the case of the solubility of caffeine in the liquid solvents [30,31], the solubility of caffeine in CO_2 with ethyl lactate cosolvent is higher than in the case of ethanol or ethyl acetate cosolvents.

Global caffeine yield (X_o) was assessed on the basis of the maximum yield attained in each OEC; 1% above the total amount of extract for each experiment as reported in Table 4. The extractable

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Table 3
Caffeine recovered (mg of caffeine in the extract/mg of caffeine in green tea leaves × 100) at different extraction times corresponding to the static-SFE of green tea leaves using different cosolvents. Extraction conditions: 30 MPa, 343 K, 210 min.

t(min)	CO_2	*t*(min)	CO_2 + EL	*t*(min)	CO_2 + ETOH	*t*(min)	CO_2 + EAC
14	3.1	14	24.3	14	14.4	14	4.9
60	15.6	37	29.6	47	28.5	37	10.5
120	19.6	86	41.0	71	32.4	96	17.8
180	21.3	210	48.3	94	34.3	210	24.6
210	21.9	–	–	148	38.1	–	–
–	–	–	–	210	40.2	–	–

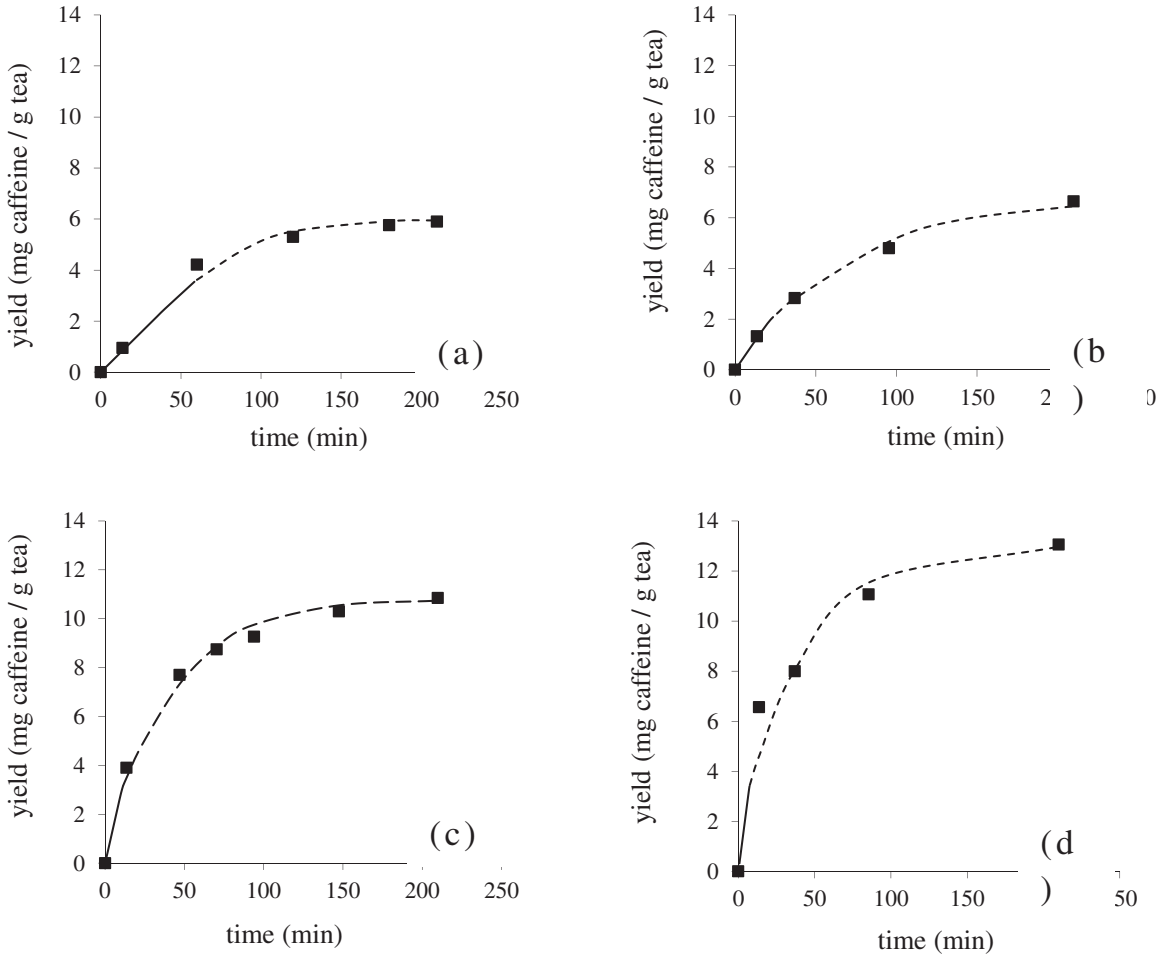

Fig. 1. Sovová's model fitting of the overall extraction curves obtained in the static-SFE of caffeine from green tea leaves at 30 MPa and 343 K. (a) CO2; (b) CO2 + EAC; (c) CO2 + ETOH; (d) CO2 + EL. Dashed lines: falling rate period.

Table 4
Parameters of the model of Sovová corresponding to the green tea leaves static-SFE at 30 MPa, 343 K and using different cosolvents.

	Y^a (kg/kg)	X_o (kg/kg)	k_{YA} (s^{-1})	k_{XA} (s^{-1})	AARD[a]
CO_2	0.00029	0.0060	0.025	0.00026	6.67
CO_2 + EAC	0.00043	0.0067	0.025	0.00013	3.72
CO_2 + ETOH	0.00134	0.0109	0.045	0.00019	2.48
CO_2 + EL	0.00220	0.0132	0.065	0.00017	8.92

[a] Absolute average relative deviation = $\frac{100}{N}\sum\frac{|Yexp-Ycal|}{Yexp}$.

caffeine (X_p) was considered an adjustable parameter, but the same X_p/X_o ratio was considered for all OECs, since the same vegetal material (crushing and cell disruption) was employed and thus, it is presumed that the same percentage of caffeine is easy accessible in the constant rate period. Then, the X_p/X_o ratio and the mass transfer coefficients in the fluid and solid phase (k_{YA} and k_{XA}) were adjusted to reproduce the experimental OECs.

A suitable X_p/X_o ratio resulted to be 0.2 and the optimal k_{YA} and k_{XA} were adjusted to each kinetic data set. The values are given in Table 4 together with the average absolute relative deviation between calculated and experimental caffeine yield.

The optimal X_p/X_o ratio represents a X_k (amount of caffeine inside cell walls) around 4 times higher than X_p (readily accessible caffeine), denoting that caffeine is strongly bond in the vegetal

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matrix. Furthermore, this is in accordance with the fact that a very short constant extraction rate period was observed in the extraction curves (t_{CER} values were 0.96, 0.16 and 0.30 min, respectively, for EA, EL and ETOH) and thus, mass transport within the solid phase dominated extraction rate almost from the beginning of the process. In this respect, the solubility value (Y^*) calculated as the slope of a theoretical linear behavior up to 14 min of extraction should be considered as an estimated value.

The quality of the model regression can be observed in Fig. 1a–d, in which the falling extraction rate period (FER) is depicted with dashed lines. A comparison of the OECs corresponding to the different supercritical fluids shows that if no cosolvents are employed the extraction entered the FER period at 54.5 min. Using the cosolvents, the caffeine extraction rate is considerably higher at the beginning of the extraction and thus, the FER period started at 23.6 min for CO_2 + EAC, 11.3 min for CO_2 + ETOH and 7.5 min for CO_2 + EL.

The solid phase mass transfer coefficients (k_{XA}) resulted rather similar in the fitting of all OECs (see Table 4). In general, k_{XA} values were two orders of magnitude lower than k_{YA} values, indicating a strong limitation of caffeine mass transfer in the solid phase. This limitation become evident from the beginning of the extraction and thus, it is possible that the saturation of the supercritical phase was not attained. Furthermore, k_{XA} values resulted reasonably similar for all cosolvents used, representing similar mass transfer in the solid phase. Regarding fluid phase mass transfer coefficient (k_{YA}), values were 1.8 and 2.6 times higher for, respectively, CO_2 + ETOH and CO_2 + EL, with respect to pure CO_2 or CO_2 + EA.

4. Conclusions

The SFE of green tea leaves was studied at 343 K and 30 MPa, using pure CO_2 and different green cosolvents for food processing. In comparison with ethyl acetate and ethanol cosolvents, ethyl lactate resulted in superior capacity for caffeine extraction. The highest yields of caffeine were obtained with ethyl lactate both in SFE-static mode (the cosolvent soaking the vegetal material before CO_2 pumping) and SFE-dynamic mode (the mixture of CO_2 + cosolvent was continuously pumped into the extraction cell). The general trend of cosolvent effect was ethyl lactate > ethanol > ethyl acetate, which corresponds with the behaviour observed in the pressurized liquid extraction of caffeine from green coffee beans [32].

The analysis of the overall extraction curves obtained in the SFE-static approach indicate that extraction velocity in the early extraction stages is around 7 times higher using CO_2 + EL than with pure supercritical CO_2. Consequently, the convective mass transfer coefficients resulted for the model of Sovová, indicate the highest value for the CO_2 + EL supercritical solvent ($k_{YA} = 6.5 \times 10^{-2}\ s^{-1}$) around 2.6 times higher than for supercritical CO_2.

Thus, ethyl lactate is a suitable green alternative to be employed as cosolvent in SFE to remove caffeine from natural matrices, reducing the extraction time and/or the amount of CO_2 employed.

Acknowledgements

D. Villanueva acknowledges the predoctoral contract (JAE) given by the Consejo Superior de Investigaciones Científicas (CSIC) of Spain. This work was financed thanks ALIBIRD, S2013/ABI-2728 (Comunidad de Madrid) project.

Appendix A.

The mass extracted (m) as a function of extraction time (t) is calculated according to the following equations:

Constant extraction rate period:

$$m = QY * [1 - \exp(-Z)]\, t \tag{1}$$

Falling extraction rate period:

$$m = QY * [t - t_{CER} \exp(Z_w - Z)] \tag{2}$$

Diffusion controlled extraction rate period:

$$m = m_{SI}\left\{X_o - \frac{Y*}{W}\ln\left[1 + \left[\exp\left(\frac{WX_o}{Y*}\right) - 1\right]\exp\left[\frac{WQ(t_{CER} - t)}{m_{SI}}\right]\left(\frac{X_k}{X_o}\right)\right]\right\} \tag{3}$$

where:

$$Z = \frac{m_{SI} k_{YA} \rho}{Q(1-\epsilon)\rho_s} \tag{4}$$

$$W = \frac{m_{SI} k_{XA}}{Q(1-\epsilon)} \tag{5}$$

$$Z_W = \frac{ZY*}{WX_o}\ln\left\{\frac{X_o \exp\left[WQ(t - t_{CER})/m_{SI}\right] - X_k}{X_o - X_k}\right\} \tag{6}$$

$$t_{CER} = \frac{m_{SI}(X_o - X_k)}{Y * ZQ} \tag{7}$$

$$m_{SI} = X_o F \tag{8}$$

Being ϵ the bed porosity, F the mass of solid loaded in the extraction cell, ρ_s the solid density, Q the CO_2 mass flow rate, ρ the CO_2 density, Y^* the solubility of the solute CO_2 and X_o the global yield.

Model parameters which are adjusted according to the experimental OEC are the intra-particle solute ratio (X_k) and the fluid phase and solid phase mass transfer coefficients (k_{YA} and k_{XA}).

Eq. (7) defines the time at which the constant extraction rate ends (t_{CER}). The time at which the falling extraction rate period begins (t_{FER}) is given by:

$$t_{FER} = t_{CER} + \frac{m_{SI}}{WQ}\ln\left[\frac{X_k + X_p \exp\left(WX_o/Y^*\right)}{X_o}\right] \tag{9}$$

References

[1] X. Wan, D. Li, Z. Zhang, Green tea and black tea. Manufacturing and consumption, in: C.T. Ho, J.K. Lin, F. Shahidi (Eds.), Tea and Tea Products. Chemistry and Health-promoting Properties, CRC Press, Boca Raton, USA, 2009, pp. 1–8.
[2] U.H. Engelhardt, Chemistry of tea, in: L. Mander, H.B. Liu (Eds.), Comprehensive Natural Products II: Chemistry and Biology, 3, Elsevier Applied Science Publishers Ltd, London, 2010, pp. 999–1032.
[3] T.R. Hartley, B.H. Sung, G.A. Pincomb, T.L. Whitsett, M.F. Wilson, W.R. Lovallo, Hypertension risk status and effect of caffeine on blood pressure, Hypertension 36 (2000) 137–141.
[4] A. Nehlig, J. Daval, G. Debry, Caffeine and the central nervous system: mechanism of action, biochemical, metabolic and psychostimulant effects, Brain Res. Rev. 17 (1992) 139–170.
[5] M. Giannellia, P. Doyle, E. Roman, M. Pelerin, C. Hermon, The effect of caffeine consumption and nausea on the risk of miscarriage, Paediatr. Perinat. Epidemiol. 17 (2003) 316–323.
[6] E. Lynge, A. Anttila, K. Hemminki, Organic solvents and cancer, Cancer, Causes Control 8 (1997) 406–419.
[7] A.B.A. de Azevedo, P. Mazzafera, R.S. Mohamed, S.A.B. Vieira de Melo, T.G. Kieckbusch, Extraction of caffeine, chlorogenic acids and lipids from green coffee beans using supercritical carbon dioxide and co-solvents, Braz. J. Chem. Eng. 25 (2008) 543–552.
[8] R.S. Mohamed, M.D.A. Saldaña, P. Mazzafera, Extraction of caffeine, theobromine, and cocoa butter from Brazilian cocoa beans using supercritical CO_2 and ethane, Ind. Eng. Chem. Res. 41 (2002) 6751–6758.
[9] C.B. Mehr, R.N. Biswal, J.L. Collins, Supercritical carbon dioxide extraction of caffeine from Guaraná, J. Supercrit. Fluids 9 (1996) 185–191.
[10] M.D.A. Saldaña, R.S. Mohamed, P. Mazzafera, Supercritical carbon dioxide extraction of methylxanthines from Maté tea leaves, Braz. J. Chem. Eng. 17 (2000) 251–260.
[11] G.I. Burgos-Solórzano, J.F. Brennecke, M.A. Stadtherr, Solubility measurements and modeling of molecules of biological and pharmaceutical interest with supercritical CO2, Fluid Phase Equilib. 220 (2004) 57–69.
[12] Zosel, Process for the decaffeination of coffee, US Patent 4247570.

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[13] S. Peter G. Brunner Process for decaffeinating coffee, US Patent 4322445.
[14] S.N. Katz, J.E. Spence, M.J. OíBrien, R.H. Skiff, G.J. Vogel, R. Prasad, Decaffeination of coffee, EU Patent 0424579B1.
[15] O.G. Vitzthum, A.S. Clauis, N.A. El-Hag, V.N. Kapoor, Decaffeination of fermented unfired tea, EU Patent 0167399A2.
[16] M.A. MacHugh, V.J. Krukonis, Supercritical Fluid Extraction, in: Principles and Practice, 2nd ed., Butterworth-Heinemann series in chemical engineering, Stoneham, USA, 1994, pp. 1–16.
[17] H.S. Park, H.K. Choi, S.J. Lee, K.W. Park, S.G. Choi, K.H. Kim, Effect of mass transfer on the removal of caffeine from green tea by supercritical carbon dioxide, J. Supercrit. Fluids 42 (2007) 205, 212.
[18] H.S. Park, H.J. Lee, M.H. Shin, K.W. Lee, H. Lee, Y.S. Kim, K.O. Kim, K.H. Kim, Effects of cosolvents on the decaffeination of green tea by supercritical carbon dioxide, Food Chem. 105 (2007) 1011–1017.
[19] H.S. Park, N.G. Im, K.H. Kim, Extraction behaviors of caffeine and chlorophylls in supercritical decaffeination of green tea leaves, Food Sci. Technol. 45 (2012) 73–78.
[20] W. Kim, J. Kim, J. Kim, S. Oh, Y. Lee, Selective caffeine removal from green tea using supercritical carbon dioxide extraction, J. Food Eng. 89 (2008) 303–309.
[21] C.J. Chang, K.L. Chiu, Y.L. Chen, C.Y. Chang, Separation of catechins from green tea using carbon dioxide extraction, Food Chem. 68 (2000) 109–113.
[22] Q.L. Sun, S. Hua, J.H. Ye, J.L. Lu, X.Q. Zheng, Y.R. Liang, Decaffeination of green tea by supercritical carbon dioxide, J. Med. Plants Res. 4 (2010) 1161–1168.
[23] K. Huang, J. Wu, Y. Chiu, C. Lai, C. Chang, Designed polar cosolvent-modified supercritical CO_2 removing caffeine from and retaining catechins in green tea powder using response surface methodology, J. Agric. Food Chem. 55 (2007) 9014–9020.
[24] M.R. García Risco, G. Vicente, G. Reglero, T. Fornari, Fractionation of thyme (*Thymus vulgaris* L.) by supercritical fluid extraction and chromatography, J. Supercrit. Fluids 55 (2011) 949–954.
[25] U. Kopcak, R.S. Mohamed, Caffeine solubility in supercritical carbon dioxide/co-solvent mixtures, J. Supercrit. Fluids 34 (2005) 209–214.
[26] E. Lack, T. Gamse, R. Marr, Extraction from solids, in: A. Bertucco, G. Vetter (Eds.), High Pressure Process Technology: Fundamentals and Applications, Industrial Chemistry Library, 9, Elsevier, Amsterdam, The Netherlands, 2001, pp. 378–395.
[27] M. Pelillo, B. Biguzzi, A. Bendini, T. GallinaToschi, M. Vanzini, G. Lercker, Preliminary investigation into development of HPLC with UV and MS-electrospray detection for the analysis of tea catechins, Food Chem. 78 (2002) 369–374.
[28] Y. Iwai, H. Nagano, G.S. Lee, M. Uno, Y. Arai, Measurement of entrainer effects of water and ethanol on solubility of caffeine in supercritical carbon dioxide by FT-IR spectroscopy, J. Supercrit. Fluids 38 (2006) 312–318.
[29] S. Aparicio, R. Alcalde, The green solvent ethyl lactate: an experimental and theoretical characterization, Green Chem. 11 (2009) 65–78.
[30] M.S. Manic, D. Villanueva, T. Fornari, A.J. Queimada, E.A. Macedo, V. Najdanovic-Visak, Solubilities of high-value compounds in ethyl lactate: measurements and modelling, J. Chem. Thermodyn. 48 (2012) 93–100.
[31] A. Shalmashi, F. Golmohammad, Solubility of caffeine in water ethyl acetate ethanol, carbon tetrachloride, methanol, chloroform, dichlorometane, and acetone between 298 and 323K, Latin Am. Appl. Res. 40 (2010) 283–285.
[32] D. Villanueva Bermejo, P. Luna, M. Manic, V. Najdanovic-Visak, G. Reglero, T. Fornari, Extraction of caffeine from natural matter using a bio-renewable agrochemical solvent, Food Bioprod. Process. 91 (2013) 303–309.
[33] H. Sovová, Rate of the vegetable oil extraction with supercritical CO_2. I. Modeling of extraction curves, Chem. Eng. Sci. 49 (1994) 409–414.
[34] National Institute of Standards and Technology (NIST). Available from: http://webbook.nist.gov/chemistry/fluid/
[35] A.B.A. de Azevedo, T.G. Kieckbusch, A.K. Tashima, R.S. Mohamed, Supercritical CO2 recovery of caffeine from green coffee oil: new experimental solubility data and modeling, Quim. Nova 31 (2008) 1319–1323.

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3.3. Producción de un extracto de té verde, concentrado en catequinas y bajo en cafeína

Separation and Purification Technology 148 (2015) 49–56

Contents lists available at ScienceDirect

Separation and Purification Technology

journal homepage: www.elsevier.com/locate/seppur

High catechins/low caffeine powder from green tea leaves by pressurized liquid extraction and supercritical antisolvent precipitation

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ARTICLE INFO

Article history:
Received 2 March 2015
Received in revised form 20 April 2015
Accepted 23 April 2015
Available online 1 May 2015

Keywords:
Green tea
Decaffeination
Ethyl lactate
Pressurized liquid extraction
Supercritical antisolvent process

ABSTRACT

This paper reports a method to produce a solvent-free extract from green tea leaves with high content of catechins and low content of caffeine, in two steps and using only "green" solvents. The method is based on the pressurized liquid extraction (PLE) of the green tea leaves using ethyl lactate as solvent, followed by a selective precipitation procedure using the supercritical carbon dioxide ($SCCO_2$) antisolvent (SAS) technique.

PLE was accomplished at 100 °C and 10 MPa, on the basis of a previous work. The influence of pressure (15–30 MPa) and temperature (50 °C and 70 °C) on the SAS precipitation process was experimentally studied in terms of precipitation yield, concentration of key bioactive compounds (caffeine and monomeric catechins) and total content of phenols of the precipitates. Additionally, a comparison using a different organic solvent (ethanol) for the extraction and precipitation steps is reported.

The precipitates obtained from the ethyl lactate PLE extracts were decaffeinated (giving less than 1% mass caffeine in the dry matter) and the concentration of catechins was close to 23% mass. The total phenolic content of the precipitates was up to 590 mg of gallic acid equivalents per g of precipitate, which represents an increase of up to 25% with respect to the PLE extracts.

1. Introduction

Tea is obtained from the leaves of the plant *Camellia sinensis* being green tea one of the most consumed types of tea. Historically, green tea has been consumed in Oriental countries like China and Japan, but its consumption in Europe and United States has increased in recent years due to its potential health benefits. Moreover, green tea extracts are also used as ingredients in other beverages (e.g. ready-to-drink beverages), in ice-creams, etc. Green tea leaves contain several bioactive compounds, such as methylxanthine alkaloids and phenolic compounds. Caffeine is the most abundant alkaloid in green tea, being the main responsible for the stimulating effects of tea. Caffeine content in tea leaves is usually around 2–5% mass of the dry weight [1–3]. Some adverse well-known effects derived from caffeine consumption include sleep deprivation, rise in blood pressure, tachycardia, abortion and miscarriages, depending on the intake concentration [4–6].

Regarding phenolic compounds, green tea is a very rich source of polyphenols (up to 30% mass of tea solids) and the major phenolic compounds are catechins [7–9]. Moreover, the main green tea catechins (flavan-3-ols) are epicatechin (EC), epicatechin gallate (ECG), epigallocatechin (EGC) and epigallocatechin gallate (EGCG), usually being EGCG the principal catechin present in green tea leaves (50–80% of total catechins in tea) [10].

Besides the contribution of catechins to tea taste, important pharmacological properties have been associated to their consumption, including antioxidant [11], anticancer [10,12], anti-inflammatory [13], antiaging [14], anti-hypercholesterolemic activity [15], antibiotic and antiviral effects [16,17].

Due to these beneficial properties of catechins and the adverse effects of caffeine, several methods have been studied to produce green tea extracts with high catechin concentration but free of caffeine. Chlorinated solvents, such as chloroform, have been used to isolate catechin compounds free from caffeine by sequential fractionation of aqueous tea extracts [18]. Chlorinated solvents are effective solvents to extract caffeine but their use has been severely reduced owing to its toxic solvent residues.

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http://dx.doi.org/10.1016/j.seppur.2015.04.037
1383-5866/

Alternatively, Dong et al. [19] used ethyl acetate (a so-called "green" solvent, of low environmental impact [20]) to isolate catechins from an aqueous green tea extract and the extract was subsequently treated with citric acid solution to remove the caffeine.

Other methods involve fractionation of a green tea extract by selective precipitation of catechins with a precipitation agent [21] and different adsorption separation processes. Several adsorbents have been tested, such as lignocellulose [22,23] and lignocellulose copolymerized with N-vinylpyrrolidone [24], activated carbon [25], polyvinylpolypyrrolidone [26,27], polyamide [28], poly(acrylamide-co-ethylene glycol dimethacrylate) [29] and different macroporous polymeric resins [30,31].

Despite the fact that substantial amounts of caffeine may be removed, many steps are needed starting from the tea leaves, large quantities of solvents are used and the loss of catechins in some cases is appreciable. Moreover, a final additional step is needed in all cases to remove the solvent and produce a dry product ready to use.

On the other hand, supercritical carbon dioxide ($SCCO_2$) has been used as antisolvent to precipitate food ingredients that are initially dissolved in organic solvents [32–35]. The solute is usually a single compound and the SAS technique is applied with the aim to produce particles of controlled morphology. In the supercritical antisolvent (SAS) technique, the liquid solvent and the antisolvent are miscible, while the solute is not soluble in the supercritical mixture. Upon contact, CO_2 removes the organic solvent from the liquid mixture, leaving a dry solute behind. Precipitation is dictated among other things by the phase behavior of the system, which is a reflection of solute–solvent–antisolvent interactions. Thus, if the initial solution is not of a single compound but a mixture, it is possible that not all solutes precipitate at the same process conditions. In this case, controlling particle morphology is less relevant, while the principle of selective precipitation can be exploited as a way to enrich the precipitate in the compounds of interest. This is a powerful idea that leads to reduced number of steps in purification and drying procedures. The concept has been applied by several authors in the fractionation of natural products [36–40], although it has not been reported for the decaffeination of tea extracts.

In the case of green tea, Sosa et al. [41] used $SCCO_2$ as antisolvent to precipitate catechins from an extract in the presence of an encapsulating agent. The extract had been previously obtained by microwave-assisted extraction using acetone as the solvent. The aim of the work was not to enrich the extracts in a particular compound but to evaluate the encapsulation efficiency. Nevertheless, the authors mentioned that around 13% of the extracted caffeine was present in the precipitate while more than 90% of the EC and ECG was recovered for one of the experiments, which evidences that the precipitate had been enriched in catechins. However, not enough data was presented to evaluate the selectivity of the extraction, precipitation and the quality of the final product.

In a similar line of research, we have recently reported the use of ethyl lactate for the selective decaffeination of green tea leaves [42] by pressurized liquid extraction (PLE). Ethyl lactate was selected as extractive solvent since it is a bio-renewable agrochemical solvent, environmental benign and permitted by the U.S. Food and Drug Administration (FDA) as pharmaceutical and food additive. The extracts obtained by this method contained both catechins and caffeine, but the extraction was proven to be selective toward caffeine indicating that caffeine is more soluble than catechins in ethyl lactate. Based on these results we speculated that a mixture ethyl lactate/CO_2 would dissolve caffeine more than catechins and lead to selective precipitation. The aim of this work was to evaluate the use of the SAS technique employing $SCCO_2$ as antisolvent to precipitate catechins from an ethyl lactate extract while removing caffeine and the organic solvent. For comparison, data using ethanol instead of ethyl lactate is also reported. The selectivity of the process is discussed in terms of precipitation yield and catechins/caffeine mass ratio of the precipitates.

2. Material and methods

2.1. Samples and reagents

"Gunpowder" green tea (*C. sinensis*) leaves were acquired in a Spanish market and were ground in a cooled knife mill using liquid nitrogen (particle size smaller than 250 μm).

Ultrapure CO_2 was provided by Air Products (Bochum, Germany). Ethyl lactate (99% purity) was obtained from Alfa-Aesar (Ward Hill, MA, USA). Acetonitrile (HPLC grade) and phosphoric acid (⩾98% purity) were obtained from Merck (Darmstadt, Germany) and ethanol (99.7% purity) from Solveco AB (Stockholm, Sweden). Folin–Ciocalteu reagent was purchased from Sigma–Aldrich (St. Louis, MO, USA).

Standards: (-)-epigallocatechin (⩾95% purity), (-)-epicatechin gallate (⩾97.5% purity) and (-)-epigallocatechin gallate (⩾98% purity) were purchased from Extrasynthèse (Genay, Lyon, Francia). (-)-epicatechin (⩾90% purity) and caffeine (⩾99% purity) were from Sigma–Aldrich.

The total content of caffeine and catechins in the vegetal material was measured using an exhaustive method as reference [2]. For this purpose, 200 mg of green tea leaves were extracted at 70 °C with 20 mL of an aqueous ethanol solution (30% v/v) in a Stuart Orbital S150 shaker apparatus (Bibby Scientific Limited Stone, UK) during 4 h. Then, the solvent was renewed and successive extraction cycles of 4 h were accomplished until the extraction yield in the corresponding cycle was lower than 2% of total yield. The content of caffeine and catechins determined by HPLC was, respectively, 22.4 mg and 82.5 mg per g of green tea leaves.

Even though this extraction method has very likely induced degradation of the catechins to a certain extent [43], it was chosen as it is similar to the ISO standard procedure for the determination of total content of catechins in green tea leaves [44] and to other exhaustive methods reported in literature [1,45,46]. Furthermore, we carried out extractions at the conditions described in the present paper using different extraction times (0.5 h, 1 h, 2 h y 4 h) and we observed the highest caffeine and catechins extraction yield after 4 h.

2.2. Pressurized liquid extraction

Extractions were carried out in an Accelerated Solvent Extraction system ASE 200 from Dionex Corporation (Sunnyvale, CA, USA).

Based on previous results [42], PLE assays with ethyl lactate and ethanol were carried out at 10 MPa and 100 °C, considering that this temperature ensures minimal thermal degradation of catechins while providing high concentration of catechins in the extract. The solid vegetal sample was dispersed with sea sand (vegetal material/sand ratio = 1 g/g).

The experimental procedure was as follows: the cells employed (11 mL capacity) were filled with the corresponding amount of sample and dispersant and automatically placed in an oven. Each cell was filled with the solvent up to the set pressure and was heated-up to the desired temperature. Then, a batch extraction was carried out for 20 min and afterwards the cell was washed with fresh solvent. The solvent was subsequently purged using nitrogen gas until the complete depressurization of the system. The extracts produced were stored under refrigeration until use.

The extracted amount of solutes is expressed as mg of dry extract per g of dry tea leaves. In Table 1, the concentration values

Table 1
Fraction extracted (mg compound in extract/mg compound in dry tea leaves), extraction yield (mg compound/g of dry tea leaves) and concentration (% mass) of key bioactive compounds in the extract of green tea leaves obtained by PLE using ethyl lactate and ethanol as extraction solvents. Temperature: 100 °C. Extraction time: 20 min.

Solvent	Compound	Concentration (% mass)	Extraction yield (mg/g)	Fraction extracted (%)
Ethyl lactate	Caffeine	12.2	16.9	75
	EGC	2.6	3.7	28
	EC	1.9	2.6	
	EGCG	9.0	12.5	
	ECG	3.3	4.6	
Ethyl lactate[a]	Caffeine	12.8	14.1	63
	EGC	4.5	4.9	28
	EC	2.0	2.1	
	EGCG	10.7	11.8	
	ECG	3.5	3.9	
Ethanol	Caffeine	10.0	20.6	92
	EGC	5.8	12.0	66
	EC	2.0	4.1	
	EGCG	14.4	29.8	
	ECG	4.0	8.3	

[a] Extract used for the replicates.

are given in percentage and they are calculated as concentration of each compound according to the respective calibration curves (mg of compound/mL) divided by the concentration of solutes in the extract (mg extract/mL). The extraction yield for each compound is expressed as mg of compound per g of dry tea leaves. The fraction extracted is expressed in percentage and it has been calculated as the amount of caffeine and catechins extracted respect to the amounts present in the dry tea leaves, according to the reference method (see Section 2.1).

2.3. SAS process

The SAS equipment (see Fig. 1) consists of two lines connected to the precipitator vessel for supplying, respectively, the CO_2 and the green tea extract. The CO_2 is pumped through the line by a syringe pump (Isco 260D. Teledyne Technologies Inc., NE, USA) and the liquid solution was pumped with a HPLC pump (Waters 515 HPLC pump. Waters Corporation, MA, USA). The precipitator vessel consists of a stainless steel vessel of 75 mL volume (Thar CL 1039. Thar Technologies, Inc. PA, USA) placed in a GC-oven (HP 5890 GC. Hewlett-Packard Co. CA, USA). The precipitator is equipped with two concentric tubes connected at the top of the precipitator vessel, the inner tube for the injection of the extract (1/32 in. tube, 160 mm length measured from valve V4, with an inner diameter of 0.180 mm) and the external tube for the CO_2 injection. A porous metallic frit (0.5 μm in diameter) is located at the bottom of the precipitator to collect the precipitate. The CO_2 flow was controlled manually by a micro-metering valve. This valve and the outlet tube were heated with hot air to prevent plugging.

CO_2 was pumped into the precipitation vessel until the pressure and temperature conditions were attained. Then, a specific amount of PLE extract was pumped into the precipitator and once the extract fed ended, additional CO_2 was pumped during 15 min to wash out the residual solvent from the precipitator. Finally, the precipitation vessel was depressurized and the particles retained in the frit were collected.

Precipitations were carried out at pressures in the range 10–30 MPa and two different temperatures (50 °C and 70 °C). The lowest pressure was the minimum needed to produce solvent-free powders. It was selected based on preliminary results in which the absence of residual solvent was confirmed by gas chromatography. The CO_2/extract flow ratios were 40 and 20 mL/mL for the ethyl lactate and ethanol extracts, respectively. The precipitations with the ethyl lactate PLE extract performed at 30 MPa, and at 50 and 70 °C, respectively, were repeated with a new extract to estimate the reproducibility of the combined steps, i.e. extraction–precipitation (entries marked with an asterisk in Tables 1–3).

All samples obtained were protected from light and stored in a freezer at -20 °C until further analysis.

In Tables 2 and 3, the precipitation yield is given in percentage and calculated as mg of particles obtained (by weighing) per mg of PLE extract supplied (calculated from the mL of extract supplied and the concentration of solutes in the extract). The concentration values are given in percentage and they are calculated as

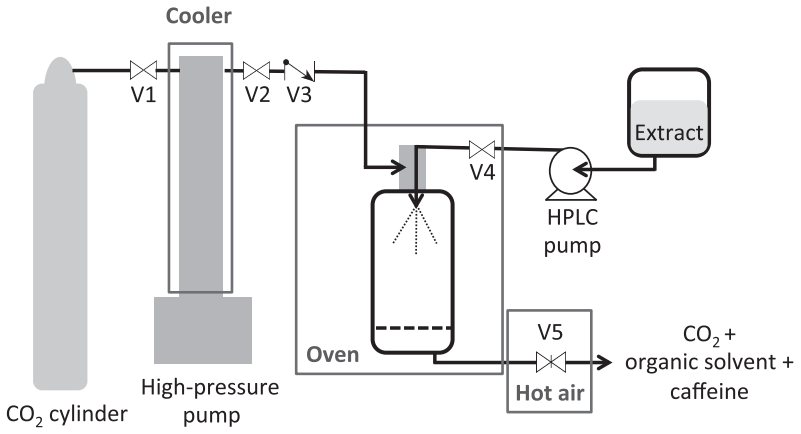

Fig. 1. Schematic diagram of the SAS process. V1, V2, V4 are on/off valves, V3 is a check-valve, V5 is a micro-metering valve.

Table 2
Effect of pressure in the SAS precipitation at 50 °C of green tea extracts obtained by PLE with ethyl lactate or ethanol. *Y*: precipitation yield (mg particles/mg PLE extract supplied); *C*: concentration (% mass in precipitate); FP: fraction precipitated (mg in precipitate/mg in PLE extract); *S*: enrichment factor (% mass in precipitate/% mass in PLE extract).

	P (MPa)	*Y* (%)	Compound	*C* (% mass)	FP (% mass)	*S*
Ethyl lactate	15	64.0	Caffeine	1.35	7.09	0.111
			EGC	3.11	75.2	1.18
			EC	2.30	79.0	1.23
			EGCG	11.1	78.3	1.22
			ECG	4.12	79.1	1.24
	20	42.5	Caffeine	1.17	4.08	0.0960
			EGC	3.22	51.7	1.22
			EC	2.37	54.0	1.27
			EGCG	11.4	53.8	1.27
			ECG	4.26	54.4	1.28
	25	59.5	Caffeine	1.11	5.42	0.0910
			EGC	3.34	75.2	1.26
			EC	2.45	78.4	1.32
			EGCG	11.9	78.1	1.31
			ECG	4.42	79.0	1.33
	30	60.8	Caffeine	1.02	5.12	0.0842
			EGC	3.36	77.2	1.27
			EC	2.47	80.6	1.33
			EGCG	11.9	80.2	1.32
			ECG	4.49	82.0	1.35
[a]	30	58.8	Caffeine	1.36	6.24	0.106
			EGC	5.99	78.8	1.34
			EC	2.32	69.9	1.19
			EGCG	13.5	74.0	1.26
			ECG	4.78	79.2	1.35
Ethanol	10	40.3	Caffeine	4.40	17.8	0.442
			EGC	6.10	42.5	1.06
			EC	2.13	43.5	1.08
			EGCG	15.4	43.2	1.07
			ECG	4.39	43.8	1.09
	15	59.9	Caffeine	2.88	17.3	0.289
			EGC	6.49	67.2	1.12
			EC	2.25	68.3	1.14
			EGCG	16.3	67.7	1.13
			ECG	4.57	68.0	1.13
	15	57.3	Caffeine	3.23	18.6	0.325
			EGC	6.03	59.7	1.04
			EC	2.13	61.7	1.08
			EGCG	15.7	62.7	1.09
			ECG	4.45	63.3	1.10
	30	32.8	Caffeine	2.28	7.50	0.229
			EGC	6.18	35.0	1.07
			EC	2.14	35.6	1.08
			EGCG	15.9	36.2	1.11
			ECG	4.49	36.5	1.11

[a] Experiments carried out with a new extract.

concentration of each compound according to the respective calibration curves (mg of compound/mL) divided by the concentration of solutes in the extract (mg extract/mL). The values of fraction precipitated are given in percentage and are calculated as mg of each compound in the precipitate per mg of the compound in the PLE extract. The enrichment factor for each compound is calculated as concentration in the precipitate divided by concentration in the PLE extract.

2.4. Identification and quantification of caffeine and catechins

The analysis was carried out in a HPLC Agilent 1100 system (Agilent Technologies, Santa Clara, CA, USA) equipped with a UV detector. The column employed was YMC ODS-A (C-18, 250 mm × 4.0 mm and 5 μm). Based on the method of Goto et al. [47], the composition of the mobile phase was (A) mixture of water and acetonitrile (95:5) containing 0.05% (v/v) phosphoric acid, and (B) mixture of water and acetonitrile (50:50) containing 0.05% (v/v) phosphoric acid. The column was maintained at 30 °C, with a flow rate of 1.0 mL/min. The mobile phase gradient employed was as follows: from initial to 8 min 90% A, 12 min 85% A, from 12 min to 15 min 85% A, 21 min 70% A, 33 min 50% A, 40 min 30% A. The injection volume was 10 μL for each run and the detection was carried out at 205 nm. The samples were injected individually.

Table 3
Effect of pressure in the SAS precipitation at 70 °C of green tea extracts obtained by PLE with ethyl lactate or ethanol. *Y*: precipitation yield (mg particles/mg PLE extract supplied); *C*: concentration (% mass in precipitate); FP: fraction precipitated (mg in precipitate/mg in PLE extract); *S*: enrichment factor (% mass in precipitate/% mass in PLE extract).

	P (MPa)	*Y* (%)	Compound	*C* (% mass)	FP	*S*
Ethyl lactate	15	46.2	Caffeine	1.32	5.01	0.108
			EGC	2.98	52.0	1.13
			EC	2.21	54.8	1.19
			EGCG	10.7	54.8	1.19
			ECG	3.88	53.7	1.16
	20	48.3	Caffeine	0.910	3.61	0.0746
			EGC	3.02	55.1	1.14
			EC	2.21	57.3	1.18
			EGCG	10.8	58.0	1.20
			ECG	4.04	58.5	1.21
	25	55.4	Caffeine	0.870	3.98	0.0718
			EGC	3.41	71.4	1.29
			EC	2.48	73.8	1.33
			EGCG	12.3	75.3	1.36
			ECG	4.59	76.2	1.38
	30	54.2	Caffeine	0.820	3.66	0.0676
			EGC	3.50	71.7	1.32
			EC	2.54	74.0	1.36
			EGCG	12.6	75.7	1.40
			ECG	4.68	76.1	1.40
[a]	30	54.4	Caffeine	1.09	4.61	0.0847
			EGC	6.41	78.0	1.43
			EC	2.50	69.4	1.28
			EGCG	14.7	74.7	1.37
			ECG	5.37	82.3	1.51
Ethanol	15	27.6	Caffeine	3.61	10.0	0.363
			EGC	6.13	29.3	1.06
			EC	2.16	30.2	1.09
			EGCG	15.7	30.1	1.09
			ECG	4.44	30.4	1.10
	30	60.4	Caffeine	2.48	15.1	0.249
			EGC	6.29	65.7	1.09
			EC	2.17	66.5	1.10
			EGCG	16.0	67.1	1.11
			ECG	4.51	67.6	1.12

[a] Experiments carried out with a new extract.

2.5. Total phenolic content

The total phenolic content in the particles and in the ethyl lactate PLE extract was determined using the Folin–Ciocalteu colorimetric method [48]. The results were expressed as GAE (mg of gallic acid/g of sample). 3 mL of distilled water was mixed with 50 μL of sample or standard, using the same concentration for the particles as for the PLE extracts. Then, 250 μL of Folin–Ciocalteu reagent was added and the content of the tube was mixed thoroughly. After 3 min, 750 μL of Na_2CO_3 (20% mass) followed by 950 μL of distilled water was added and the mixture was allowed to stand for 2 h at ambient conditions. The absorbance was measured at 760 nm in a Multiskan GO microplate spectrophotometer reader (Thermo Fisher Scientific, Waltham, USA). The measurements were carried out in triplicate for each precipitate.

2.6. Scanning electron microscopy (SEM)

The particles collected in the precipitator vessel were observed in a JSM 6700F (Jeol Ltd., Tokyo, Japan) scanning electronic microscope (SEM), after coating of the samples with a thin gold layer by a SCD 004 sputter coater (Oerlikon Balzers, Balzers, Liechtenstein).

3. Results and discussion

Natural extracts like the ones obtained from green tea are complex mixtures as they contain a large number of compounds of different chemical properties. The solubility of tea-derived compounds naturally varies in different solvents. The solvent power of $SCCO_2$ can be tuned by changes in pressure and temperature, as well as by adding different amounts of an organic solvent. Therefore, while some tea-derived compounds will be soluble, others might precipitate depending on the pressure, temperature, and amount of organic co-solvent, of which the latter in a SAS process is represented by the CO_2/extract flow ratios. Ethyl lactate and ethanol are both considered green solvents with quite different physical properties (density, dipole moment, dielectric constant, etc.). It was expected that different phase behavior in ethyl lactate/CO_2 and ethanol/CO_2 mixtures would lead to a solvent-free product with different compositions. Pressure, temperature and CO_2/extract flow ratios have been set experimentally within a range of values that enable complete removal of the organic solvent. The ranges differ for ethanol and ethyl lactate due to their different physical properties.

All these aspects are evaluated in the following sections in relation to producing a precipitate with the highest possible catechins/caffeine ratio at the highest possible yields.

3.1. PLE of green tea leaves

The extracted amount of solutes obtained with ethyl lactate was 138 and 110 mg of extract per g of dry tea leaves (first batch and replicate, respectively) and the obtained amount with ethanol was 207 mg of extract per g of dry tea leaves.

Table 1 shows the fraction extracted, extraction yield and concentration of caffeine and main catechins obtained in the PLE extracts produced using ethyl lactate and ethanol as extraction solvents. The extraction yield for caffeine was higher for ethanol (20.6 mg/g) than for ethyl lactate (16.9 mg/g for the first batch and 14.1 mg/g for the replicate). The same is true for the total sum of catechins, 54.2 mg/g with ethanol versus 23.4 mg/g for the first batch with ethyl lactate and 22.7 mg/g for the replicate. The extracts obtained with ethyl lactate have a higher ratio in caffeine/catechins content, which is reflected in the concentrations and in the fraction extracted of each compound. The different composition of the extracts has to be considered when comparing the influence of a different organic solvent in the selectivity of the SAS precipitation.

In the case of ethyl lactate, the first batch was used for the SAS experiments, while the batch with an asterisk was used for SAS replicates.

3.2. SAS process

Tables 2 and 3 show the results obtained from the SAS precipitation of the ethyl lactate PLE extracts at 50 °C and 70 °C, respectively, as a function of the SAS precipitation pressure. The tables show the experimental conditions and the effect of the studied operating parameters (temperature, pressure) on the precipitation of caffeine and catechins. For comparison, the tables include data obtained using ethanol as PLE solvent. In the range of temperatures and pressures investigated, the CO_2/extract flow ratio (mL/mL) was set to 40 and 20, for ethyl lactate and ethanol extracts, respectively.

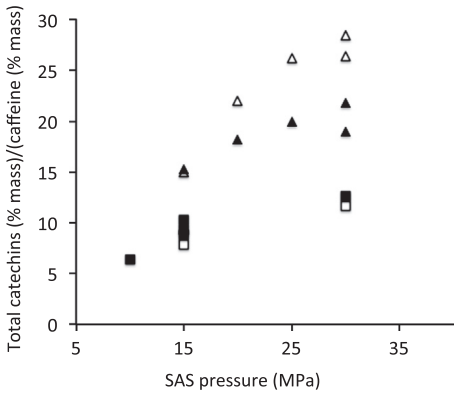

Fig. 2. Total catechins (% mass)/caffeine (% mass) ratio in the precipitates obtained from the (▲, △) ethyl lactate and (■, □) ethanol PLE extracts as a function of SAS precipitation pressure. Full symbols: 50 °C; empty symbols: 70 °C.

In all cases, the SAS process resulted in precipitates with a reduced content of caffeine, in comparison with the content of caffeine in the PLE extracts. In the case of ethyl lactate, the caffeine concentration went from 12.2% mass in the PLE extract (Table 1) to 0.8–1.4% mass in the precipitates. In other words, the caffeine reduction was in all cases higher than 89%, as it is indicated by enrichment factors above 0.11, at precipitation yields ranging between 42% and 64%. In the case of ethanol, the concentration of caffeine in the PLE extract was 10% mass (Table 1), while in the precipitates the concentrations were in the range 2.3–4.4% mass. The caffeine reduction was in all cases lower than 77%, as it is indicated by enrichment factors lower than 0.23, at precipitation yields ranging between 27% and 60%. For the range of studied parameters and with ethyl lactate, it was possible to achieve caffeine reductions as high as 93%, as it is indicated by an enrichment factor of 0.068, at precipitation yields of 54%. This remarkable reduction led to precipitates with less than 1% mass of caffeine in the dry matter, which can be considered a "decaffeinated" tea extract by European regulations [49].

With respect to the catechins and in the case of the precipitates produced from the ethyl lactate PLE extracts, the concentration of EGCG (the main catechin identified) increased from 9% mass in the extract to values between 10.7% and 12.6% mass in the precipitates, which corresponds to enrichment factors from 1.19 to 1.40 respectively at precipitation yields ranging between 42% and 64%. In the case of ethanol, the maximum increase in concentration of EGCG was from 14.4% mass in the extract to 16.3% mass in the precipitate, which corresponds to an enrichment factor not higher than 1.13 at precipitation yields of 54%. For both solvents it was possible to obtain precipitates with 29% mass of total catechins.

Selectivity of the precipitation toward a particular compound is a function of precipitation yield. As can be seen in Tables 2 and 3,

Table 4
Total phenolic content expressed as mg of gallic acid equivalents (GAE)/g of precipitate in the particles obtained by SAS precipitation of ethyl lactate PLE extract. mg of GAE/g of dry extract in ethyl lactate PLE extract = 470 ± 16.

P (MPa)	CO_2/extract (mL/mL)	GAE (mg of gallic acid equivalents/g of precipitate)	
		50 °C	70 °C
15	40	570 ± 24	530 ± 9
20	40	556 ± 26	545 ± 16
25	40	556 ± 5	567 ± 17
30	40	590 ± 8	578 ± 14

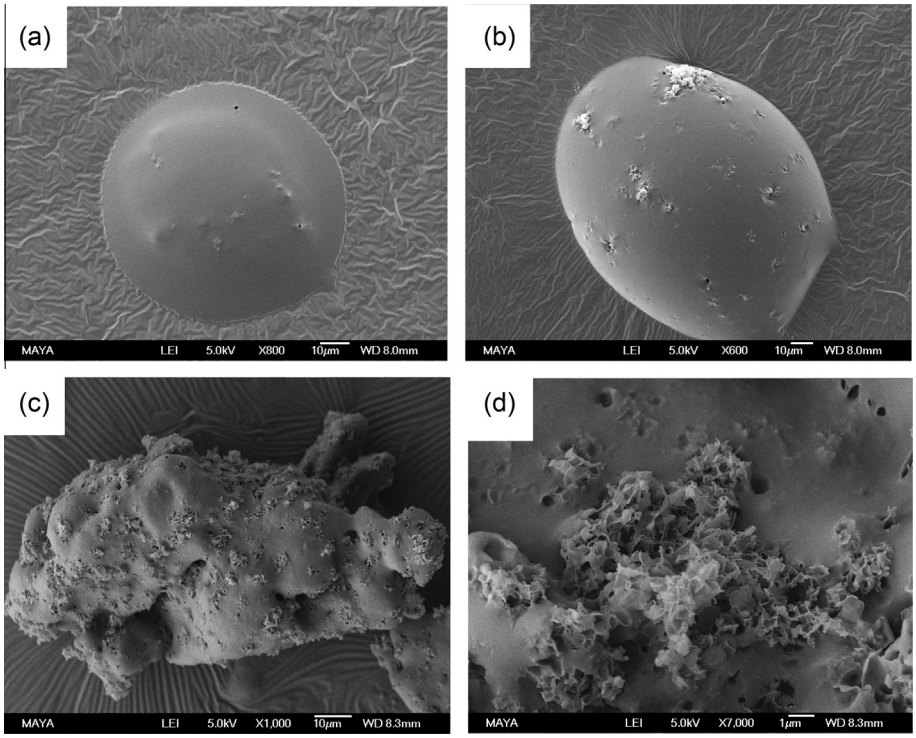

Fig. 3. SEM images of particles obtained from the ethyl lactate extract by SAS at 20 MPa and 70 °C (a), 30 MPa and 70 °C (b), and 30 MPa and 50 °C (c and d).

precipitation yields differ for different precipitation parameters. Therefore, it is necessary to be very careful when comparing different entries in the tables. It is only possible to state that certain process parameters lead to a more selective precipitation than others if both the catechins/caffeine content and the precipitation yield are higher. Fig. 2 shows the total catechin (% mass)/caffeine (% mass) ratio of the precipitates from ethyl lactate and ethanol, as a function of the SAS pressure and temperature. It is possible to visualize that using ethyl lactate leads to precipitates with values of catechins/caffeine ratios between 1.5 and 2.3 times higher than for ethanol. At the same time, the average precipitation yield was around 10% higher with ethyl lactate. Regarding the fraction of catechins precipitated (mg in precipitate/mg in PLE extract), the highest values were obtained from the ethyl lactate PLE extracts (up to 80.2% for EGCG, the main catechin extracted) versus ethanol PLE extracts (up to 67.7% for EGCG). In the case of the caffeine, the lowest values of fraction precipitated were obtained with ethyl lactate under all studied conditions, in the range of 7.1–3.6%, while for ethanol the fraction of caffeine precipitated was in the range of 18.0–7.5%.

These results are strong indications that the extraction–precipitation process with ethyl lactate is more selective than that with ethanol. This is more dramatic if we consider that the ratio between caffeine and catechins in the initial extracts was much higher for ethyl lactate than for ethanol. A possible explanation for this selectivity can be made in terms of polarity. Catechins have more affinity for the mixture ethanol/CO_2 than for ethyl lactate/CO_2. This is probably because catechins are quite polar compounds and ethanol is a more polar solvent than ethyl lactate. Therefore, catechins tend to be partially soluble in ethanol/CO_2 and they exit the precipitation chamber through the vent instead of precipitating during the SAS process, while they are less partially soluble in the case of ethyl lactate/CO_2 and precipitate in a bigger extent. The opposite is true for caffeine. Since caffeine is a less polar compound it shows more affinity for the ethyl lactate/CO_2 mixture and it tends to be more partially soluble than for ethanol/CO_2, which explains why it is removed from the ethyl lactate PLE extracts in a bigger extent.

In all cases, increasing pressure has a tendency to increase the catechins/caffeine ratio (see Fig. 2), which becomes more evident for ethyl lactate at the highest temperature. Furthermore, temperature seems to make a bigger impact in the case of ethyl lactate. 70 °C led to the highest catechins/caffeine ratios while maintaining an average precipitation yield only five units lower than for 50 °C.

3.3. Total phenolic content

Table 4 shows the total phenolic content of the precipitates obtained from the ethyl lactate PLE extracts. The phenolic content is referred as mg of gallic acid equivalents (GAE) per g of dry sample. Table 4 shows average values of the triplicates. The mean standard deviation was lower than 15 mg GAE/g dry sample in all cases. High content of phenolic compounds were obtained in the precipitates, with values in the range 530–590 mg GAE/g precipitate. Taking into account the phenolic content in the PLE extract (470 ± 16 mg GAE/g dry extract), the increase of total phenolic content was around 12–25%.

3.4. Particle morphology

The color of the precipitates was light yellow.

Fig. 3 shows images of the different morphologies observed, corresponding to particles obtained at 20 MPa and 70 °C (a), 30 MPa and 70 °C (b), and 30 MPa and 50 °C (c and d). Even though the morphology of the particles and its relation with process parameters is not relevant in this study, it is shown as a way to further characterize the precipitate and to satisfy curious readers. The particles had irregular shapes, with dimensions in the order of 100 μm and a smooth surface, sprinkled with areas of flaky appearance.

4. Conclusions

We have developed a clean method to produce decaffeinated green tea extracts of high catechins content by PLE with ethyl lactate followed by SAS precipitation. The results indicate that the combination of ethyl lactate and $SCCO_2$ as antisolvent induces selective precipitation of catechins versus caffeine, which enables in one step the removal of the solvent from the PLE extracts and the enrichment of the precipitate.

The total catechins/caffeine mass ratios obtained in the ethyl lactate precipitates were up to 2.3 times higher than those produced with ethanol, with average precipitation yields 10% higher. These results indicated that the selectivity of the precipitation achieved with ethyl lactate was higher than with ethanol.

Precipitates with less than 1% mass of caffeine, 23% mass of catechins and high content of phenolic compounds (550–580 mg GAE per g precipitate) were produced at 70 °C and 20–30 MPa, with CO_2/extract flow ratios of 40 mL/mL. This composition represents a reduction of caffeine content around 93% with respect to the original extract, and enrichment factors for EGCG (the main catechin identified) up to 1.40, which makes these particles a valuable product for food, cosmetics and pharmaceutical industries.

Acknowledgements

D. Villanueva acknowledges the predoctoral contract (JAE) given by the Consejo Superior de Investigaciones Científicas (CSIC) and the fellowship given by Universidad Autónoma de Madrid. This work was financed thanks to Comunidad Autónoma de Madrid (project ALIBIRD-S2013/ABI-2728).

Turner acknowledges the Swedish Research Council (2010-333) and the Swedish Research Council Formas (229-2009-1527). Irene Rodríguez Meizoso acknowledges The Swedish Research Council (VR, 2012-4124) for financial support.

References

[1] A. Perva-Uzunalic, M. Skerget, Z. Knez, B. Weinreich, F. Otto, S. Gruner, Extraction of active ingredients from green tea (*Camellia sinensis*): extraction efficiency of major catechins and caffeine, Food Chem. 96 (2006) 597–605.
[2] H.S. Park, H.K. Choi, S.J. Lee, K.W. Park, S.G. Choi, K.H. Kim, Effect of mass transfer on the removal of caffeine from green tea by supercritical carbon dioxide, J. Supercrit. Fluids 42 (2007) 205–212.
[3] P. Fernández, M. Martín, A. González, F. Pablos, HPLC determination of catechins and caffeine in tea. Differentiation of green, black and instant teas, Analyst 125 (2000) 421–425.
[4] A. Nehlig, J. Daval, G. Debry, Caffeine and the central nervous system: mechanism of action, biochemical, metabolic and psychostimulant effects, Brain Res. Rev. 17 (1992) 139–170.
[5] T.R. Hartley, B.H. Sung, G.A. Pincomb, T.L. Whitsett, M.F. Wilson, W.R. Lovallo, Hypertension risk status and effect of caffeine on blood pressure, Hypertension 36 (2000) 137–141.
[6] M. Giannellia, P. Doyle, E. Roman, M. Pelerin, C. Hermon, The effect of caffeine consumption and nausea on the risk of miscarriage, Paediatr. Perinat. Epidemiol. 17 (2003) 316–323.
[7] K. Wei, L. Wang, J. Zhou, W. He, J. Zeng, Y. Jiang, H. Cheng, Catechin contents in tea (*Camellia sinensis*) as affected by cultivar and environment and their relation to chlorophyll contents, Food Chem. 125 (2011) 44–48.
[8] Q. Chen, J. Zhao, X. Huang, H. Zhang, M. Liu, Simultaneous determination of total polyphenols and caffeine contents of green tea by near-infrared reflectance spectroscopy, Microchem. J. 83 (2006) 42–47.
[9] U.H. Engelhardt, Comprehensive natural products II: chemistry and biology, in: L. Mander, H.B. Liu (Eds.), Chemistry of Tea, vol. 3, Elsevier Applied Science Publishers Ltd., London, 2010, pp. 999–1032.
[10] N. Khan, H. Mukhtar, Tea polyphenols for health promotion, Life Sci. 81 (2007) 519–533.
[11] Z.Q. Liu, L.P. Ma, B. Zhou, L. Yang, Z.L. Liu, Antioxidative effects of green tea polyphenols on free radical initiated and photosensitized peroxidation of human low density lipoprotein, Chem. Phys. Lipids 106 (2000) 53–63.
[12] S.B. Moyers, N.B. Kumar, Green tea polyphenols and cancer chemoprevention: multiple mechanisms and endpoints for phase II trials, Nutr. Rev. 62 (2004) 204–211.
[13] E. Tedeschi, H. Suzuki, M. Menegazzi, Antiinflammatory action of EGCG, the main component of green tea, through STAT-1 inhibition, Ann. N.Y. Acad. Sci. 973 (2002) 435–437.
[14] R. Cooper, D.J. Morre, D.M. Morre, Medicinal benefits of green tea: Part I. Review of noncancer health benefits, J. Altern. Complement. Med. 11 (2005) 521–528.
[15] Y. Matsui, H. Kumagai, H. Masuda, Antihypercholesterolemic activity of catechin-freesaponin-rich extract from green tea leaves, Food Sci. Technol. Res. 12 (2006) 50–54.
[16] Y. Yanagawa, Y. Yamamoto, Y. Hara, T. Shimamura, A combination effect of epigallocatechin gallate, a major compound of green tea catechins, with antibiotics on *Helicobacter pylori* growth in vitro, Curr. Microbiol. 47 (2003) 244–249.
[17] P.D. Stapleton, S. Shah, J.C. Anderson, Y. Hara, J.M.T. Hamilton-Miller, P.W. Taylor, Modulation of beta-lactam resistance in *Staphylococcus aureus* by catechins and gallates, Int. J. Antimicrob. Agents 23 (2004) 462–467.
[18] K. Row, Y. Jin, Recovery of catechin compounds from Korean tea by solvent extraction, Bioresour. Technol. 97 (2006) 790–793.
[19] J. Dong, J. Ye, J. Lu, X. Zheng, Y. Liang, Isolation of antioxidant catechins from green tea and its decaffeination, Food Bioprod. Process. 89 (2011) 62–66.
[20] C.S.M. Pereira, V.M.T.M. Silva, A.E. Rodrigues, Ethyl lactate as a solvent: properties, applications and production processes – a review, Green Chem. 13 (2011) 2658–2671.
[21] M. Monsanto, N. Hooshyar, J. Meuldijk, E. Zondervan, Modeling and optimization of green tea precipitation for the recovery of catechins, Sep. Purif. Technol. 129 (2014) 129–136.
[22] S. Sakanaka, A novel convenient process to obtain a raw decaffeinated tea polyphenol fraction using a lignocellulose column, J. Agric. Food Chem. 51 (2003) 3140–3143.
[23] J.H. Ye, J. Jin, H.L. Liang, J.L. Lu, Y.Y. Du, X.Q. Zheng, Y.R. Liang, Using tea stalk lignocellulose as an adsorbent for separating decaffeinated tea catechins, Bioresour. Technol. 100 (2009) 622–628.
[24] J.H. Ye, J.J. Dong, J.L. Lu, X.Q. Zheng, J. Jin, H. Chen, Y.R. Liang, Effect of graft copolymerization of fir sawdust lignocellulose with N-vinylpyrrolidone on adsorption capacity to tea catechins, Carbohydr. Polym. 81 (2010) 441–447.
[25] J.H. Ye, Y.R. Liang, J. Jin, H.L. Liang, Y.Y. Du, J.L. Lu, Q. Ye, C. Lin, Preparation of partially decaffeinated instant green tea, J. Agric. Food Chem. 55 (2007) 3498–3502.
[26] Z.B. Dong, Y.R. Liang, F.Y. Fan, J.H. Ye, X.Q. Zheng, J.L. Lu, Adsorption behavior of the catechins and caffeine onto polyvinylpolypyrrolidone, J. Agric. Food Chem. 59 (2011) 4238–4247.
[27] F.Y. Fan, Q. Gan, Z.B. Dong, K.J. Song, X.Q. Zheng, Y.R. Liang, J.L. Lu, Selective elution of tea catechins and caffeine from polyvinylpolypyrrolidone, Int. J. Food Sci. Technol. 49 (2014) 1626–1634.
[28] J.H. Ye, L.X. Wang, H. Chen, J.J. Dong, J.L. Lu, X.Q. Zheng, M.Y. Wu, Y.R. Liang, Preparation of tea catechins using polyamide, J. Biosci. Bioeng. 111 (2011) 232–236.
[29] J.L. Lu, M.Y. Wu, X.L. Yang, Z.B. Dong, J.H. Ye, D. Borthakur, Q.L. Sun, Y.R. Liang, Decaffeination of tea extracts by using poly(acrylamide-co-ethylene glycol dimethylacrylate) as adsorbent, J. Food Eng. 97 (2010) 555–562.
[30] D. Méndez Sevillano, L.A.M. van der Wielen, N. Hooshyar, M. Ottens, Resin selection for the separation of caffeine from green tea catechins, Food Bioprod. Process. 92 (2014) 192–198.
[31] X. Zhang, S.C. Lei, S. Jabbar, B. Hu, Y. Sun, X.X. Zeng, Simultaneous separation and purification of tea bioactives from summer green tea by column chromatography, J. Chem. Soc. Pak. 35 (2013) 1258–1267.
[32] D.T. Santos, M.A.A. Meireles, Micronization and encapsulation of functional pigments using supercritical carbon dioxide, J. Food Process. Eng. 36 (2013) 36–49.
[33] J.J. Wu, C.T. Shen, T.T. Jong, C.C. Young, H.L. Yang, S.L. Hsu, C.J. Chang, C.J. Shieh, Supercritical carbon dioxide anti-solvent process for purification of micronized propolis particulates and associated anti-cancer activity, Sep. Purif. Technol. 70 (2009) 190–198.
[34] Q. Zhong, M. Jin, D. Xiao, H. Tian, W. Zhang, Application of supercritical anti-solvent technologies for the synthesis of delivery systems of bioactive food components, Food Biophys. 3 (2008) 186–190.
[35] C. Magnan, E. Badens, N. Commenges, G. Charbit, Soy lecithin micronization by precipitation with a compressed fluid antisolvent – influence of process parameters, J. Supercrit. Fluids 19 (2000) 69–77.
[36] J.L. Marqués, G. Della Porta, E. Reverchon, J.A.R. Renuncio, A.M. Mainar, Supercritical antisolvent extraction of antioxidants from grape seeds after vinification, J. Supercrit. Fluids 82 (2013) 238–243.
[37] H. Aro, E. Järvenpää, K. Könkö, M. Sihvonen, V. Hietaniemi, R. Huopalahti, Isolation and purification of egg yolk phospholipids using liquid extraction and pilot-scale supercritical fluid techniques, Eur. Food Res. Technol. 228 (2009) 857–863.
[38] M. Mukhopadhyay, S. Singh, Refining of crude lecithin using dense carbon dioxide as anti-solvent, J. Supercrit. Fluids 30 (2004) 201–211.
[39] O.J. Catchpole, J.B. Grey, K.A. Mitchell, J.S. Lan, Supercritical antisolvent fractionation of propolis tincture, J. Supercrit. Fluids 29 (2004) 97–106.
[40] M.B.O. Andersson, M. Demirbüker, L.G. Blomberg, Semi-continuous extraction–purification of lipids by means of supercritical fluids, J. Chromatogr. A 785 (1997) 337–343.
[41] M. Sosa, S. Rodríguez-Rojo, F. Mattea, M. Cismondi, M.J. Cocero, Green tea encapsulation by means of high pressure antisolvent coprecipitation, J. Supercrit. Fluids 56 (2011) 304–311.
[42] D. Villanueva Bermejo, J. Mendiola, E. Ibáñez, G. Reglero, T. Fornari, Analysis of the pressurized liquid extraction of caffeine and catechins from green tea

leaves using ethyl lactate, water and ethyl lactate + water mixtures, Food Bioprod. Process. (submitted for publication).
[43] R. Wang, W. Zhou, X. Jiang, Reaction kinetics of degradation and epimerization of epigallocatechin gallate (EGCG) in aqueous system over a wide temperature range, J. Agric. Food Chem. 56 (2008) 2694–2701.
[44] ISO, Determination of substances characteristic of green and black tea – Part 2: Content of catechins in green tea – Method using high-performance liquid chromatography. ISO 14502-2:2005, 2005.
[45] W.J. Kim, J.D. Kim, J. Kim, S.G. Oh, Y.W. Lee, Selective caffeine removal from green tea using supercritical carbon dioxide extraction, J. Food Eng. 89 (2008) 303–309.
[46] Y.S. Lin, Y.J. Tsai, J.S. Tsay, J.K. Lin, Factors affecting the levels of tea polyphenols and caffeine in tea leaves, J. Agric. Food Chem. 51 (2003) 1864–1873.
[47] T. Goto, Y. Yoshida, M. Kiso, H. Nagashima, Simultaneous analysis of individual catechins and caffeine in green tea, J. Chromatogr. A 749 (1996) 295–299.
[48] V.L. Singleton, R. Orthofer, R.M. Lamuela-Raventós, Analysis of total phenols and other oxidations substrates and antioxidants by means of Folin–Ciocalteu reagent, Methods Enzymol. 299 (1999) 152–178.
[49] Compendium of guidelines for tea. (2014, September 18). Retrieved from: <http://www.etc-online.org/docs/compendium-of-guidelines-for-tea.pdf>.

4

DISCUSIÓN

4. DISCUSIÓN

Como se ha comentado anteriormente, en la producción de alimentos funcionales es frecuente una o varias etapas relacionadas con la extracción y purificación de ingredientes con actividades biológicas a partir de fuentes naturales. En este sentido, el desarrollo de procesos que utilicen disolventes verdes, ecológicos y aceptados en la industria alimentaria, de acuerdo con los principios de la Química Verde, es un factor esencial a tener en cuenta.

El lactato de etilo es un compuesto que existe de forma natural en diversos alimentos, como vino, cerveza, carne o algunas frutas, formando parte de la fracción de los compuestos que confieren las características sensoriales. Debido a sus propiedades físico-químicas, así como a sus propiedades beneficiosas desde un punto de vista medioambiental y de seguridad, el lactato de etilo es utilizado como disolvente en varios sectores industriales, entre ellos, el sector farmacéutico y alimentario. Asimismo, actualmente se está llevando a cabo un intenso estudio relacionado con su producción, con el objetivo de desarrollar procedimientos que permitan su obtención a precios cada vez más competitivos, utilizando como materia prima subproductos procedentes de la industria agroalimentaria.

Sin embargo, hasta el momento, el lactato de etilo ha sido poco estudiado como disolvente de extracción de compuestos alimentarios bioactivos. Los trabajos publicados hasta ahora son recientes, y han sido dirigidos fundamentalmente hacia la extracción de compuestos de naturaleza lipídica (carotenoides, escualeno, tocoferoles y ácido γ-linolénico).

Con el objetivo general de aplicar el lactato de etilo como disolvente de extracción de compuestos bioactivos, en primer lugar se realizó la medición de la solubilidad en lactato de etilo de sustancias de interés comercial, resultando particularmente alta la solubilidad de cafeína en lactato de etilo. Así, se ha continuado el desarrollo de esta tesis con el estudio acerca de la aplicación del lactato de etilo como nuevo disolvente ecológico para la extracción de cafeína de matrices vegetales. En concreto se estudiaron los granos de café verde y las hojas de té verde, considerando la importancia comercial que actualmente tiene a nivel mundial el mercado de té y café y sus productos descafeinados, así como la posterior recuperación de la cafeína extraída como ingrediente en la industria alimentaria y farmacéutica. Con este objetivo, se aplicaron

tecnologías más eficientes y ecológicas que las tradicionales, tales como la extracción con líquidos presurizados y la extracción supercrítica. Finalmente, aplicando lactato de etilo y la tecnología de CO_2 supercrítico, se llevó a cabo la obtención de un extracto de té verde descafeinado y concentrado en catequinas, como ingrediente que puede ser utilizado en la elaboración de alimentos funcionales.

4.1. Medición de la solubilidad y del equilibrio de fases de sistemas que contienen lactato de etilo.

En el desarrollo de procesos de extracción, la solubilidad de los compuestos de interés en el disolvente constituye una información relevante para estimar la viabilidad del proceso. Respecto al lactato de etilo, es muy escasa la información de este tipo disponible en la bibliografía ("*Ethyl lactate: a biorenewable agrochemical solvent for food technology*" Handbook of Solvents, Volume 2, Use, Health, and Environment). En el trabajo titulado "*Solubility of high-value compounds in ethyl lactate: measurements and modeling*" (Journal of Chemical Thermodynamics, 48 (2012) 93-100) se muestran los resultados de la medida de solubilidad en lactato de etilo de diferentes compuestos bioactivos de interés: cafeína, timol, ácido cafeico, ácido ferúlico y ácido vainíllico, a diferentes temperaturas. Los principales resultados derivados de este trabajo son los siguientes:

- El timol es el compuesto que presentó la mayor solubilidad en lactato de etilo (91,8 % en peso a 45 ºC), aunque algo inferior a la que presenta en etanol, tal y como se demostró en el trabajo titulado "*Extraction of thymol from different varieties of thyme plants using green solvents*" (Journal of the Science of Food and Agriculture. DOI: 10.1002/jsfa.7031) (ver Anexo). Además, en este trabajo se demostró que, respecto a la extracción con CO_2 supercrítico de hojas de tomillo, el uso de lactato de etilo como cosolvente no mejora significativamente la recuperación de timol.
- La menor solubilidad la presentó el ácido cafeico, siendo la solubilidad medida a 60 °C de 2,6 % en peso, seguida del ácido vainíllico y ferúlico, con valores de 7,6 y 9,7 % en peso a la misma temperatura. En el caso del ácido cafeico, a diferencia del ácido ferúlico, la presencia de un grupo hidroxilo en el anillo aromático, en lugar del grupo metoxilo que presenta el ácido ferúlico en su estructura, produce una considerable disminución de la solubilidad. Del mismo modo, teniendo en cuenta las estructuras del ácido ferúlico y ácido vainíllico, se puede concluir que la

presencia de la cadena alquílica unida al grupo carboxilo del ácido ferúlico produce un incremento de la solubilidad.

- También se observó que la presencia de un pequeño porcentaje de agua (1,4 % en peso) en el lactato de etilo produce un efecto cosolvente, aumentando la solubilidad de varios de los compuestos estudiados, en concreto, cafeína, ácido ferúlico y ácido cafeico, obteniéndose solubilidades mayores que las correspondientes al lactato de etilo y agua puros. Esto se demuestra en el trabajo "*Pressurized liquid extraction of caffeine and catechins from green tea leaves using ethyl lactate, water and ethyl lactate + water mixtures*" (Food and Bioproducts Processing, 96 (2015) 106-112), donde además de las mediciones de solubilidad de cafeína a 25 °C para distintas relaciones lactato de etilo / agua, se presenta la extracción de hojas de té verde con los disolventes presurizados, resultando en un considerable aumento de la recuperación de cafeína para mezclas con un 50 % de lactato de etilo. Este efecto cosolvente también se observó en el caso del ácido ferúlico, en el trabajo *"Solubility of bioactive substances in ethyl lactate + water mixtures: experimental data and modeling"* (The Open Chemical Engineering Journal, enviado).
- Respecto a la cafeína, la solubilidad en lactato de etilo a temperatura ambiente fue similar a la solubilidad en agua y superior a la solubilidad en tetracloruro de carbono, uno de los primeros disolventes que se utilizaron a nivel comercial para el descafeinado, y a la del acetato de etilo, un disolvente obtenido mediante síntesis química, actualmente utilizado para descafeinar café y té a nivel industrial. Igualmente, la solubilidad de cafeína en lactato de etilo fue superior a la de otros disolventes orgánicos estudiados en la bibliografía para la extracción de cafeína, como son el metanol y la acetona (ver Tabla 4.1). Por el contrario, la solubilidad de cafeína en lactato de etilo es considerablemente inferior a la de los disolventes clorados cloroformo y cloruro de metileno. No obstante, como se ha comentado en secciones anteriores, la aplicación de estos disolventes tiende a eliminarse debido a su toxicidad y a los problemas medioambientales que generan.

Tabla 4.1. Solubilidad de cafeína en varios disolventes orgánicos, a 25 ºC. Lactato de etilo: obtenido experimentalmente en esta tesis. Resto de disolventes: Shalmashi y Golmohammad (2010).

Disolvente	Solubilidad (% en peso)	Disolvente	Solubilidad (% en peso)
Lactato de etilo	2,3	Acetona	1,5
Agua	2,2	Tetracloruro de carbono	0,2
Acetato de etilo	0,9	Cloruro de metileno	8,1
Metanol	1,2	Cloroformo	10,4
Etanol	0,7		

En base a estos resultados, como objetivo de esta tesis se planteó la extracción de cafeína de matrices vegetales utilizando lactato de etilo. Las matrices vegetales elegidas fueron granos de café verde y hojas de té verde, debido al interés comercial que generan sus productos descafeinados, cuya producción y consumo ha ido en aumento debido, entre otras cosas, a la posible repercusión del consumo de cafeína en enfermedades crónicas frecuentes en países occidentales, como la hipertensión. Este estudio de extracción se discute en la Sección 4.2.

Por otro lado, en el trabajo titulado "*Solubility of CO_2 in ethyl lactate and modeling of the phase behavior of the CO_2 + ethyl lactate mixture*" (Journal of Chemical and Engineering Data, 58 (2013) 301-306), se llevó a cabo el estudio de la solubilidad de CO_2 en lactato de etilo, a 38, 45 y 50 ºC, en un rango de presiones comprendido entre 1 y 10 MPa. Así, junto con los datos de solubilidad de lactato de etilo en CO_2 medidos por Chylinski y Gregorowicz (1998), fue posible definir el diagrama de fases líquido + vapor del sistema binario CO_2 + lactato de etilo.

El análisis de los datos de equilibrio permitieron comprobar experimentalmente la formación de una fase homogénea a presiones mayores de 10 MPa y a las temperaturas estudiadas, habituales en los procesos de extracción con CO_2 supercrítico, estableciendo condiciones adecuadas para utilizar el lactato de etilo como cosolvente del CO_2 en la tecnología supercrítica. Esta información experimental permitió avanzar en el estudio de la extracción supercrítica de cafeína de matrices vegetales utilizando lactato de etilo como cosolvente (Sección 4.2), así como en la precipitación supercrítica solvente-

antisolvente para producir un extracto de té verde con bajo contenido de cafeína (Sección 4.3).

4.2. Extracción de cafeína de granos de café verde y hojas de té verde utilizando lactato de etilo.

Para el estudio de la extracción de cafeína con lactato de etilo, se utilizaron dos procedimientos de extracción: la extracción sólido-líquido mediante líquidos presurizados (PLE) y la extracción supercrítica (SFE). Ambas tecnologías son reconocidas como técnicas más eficaces, más rápidas y medioambientalmente más limpias que la extracción con disolventes a presión ambiente, y han sido ampliamente estudiadas en las últimas décadas para la extracción de ingredientes alimentarios bioactivos.

4.2.1. Extracción de cafeína de granos de café verde.

En el trabajo titulado "*Extraction of caffeine from natural matter using a bio-renewable agrochemical solvent*" (Food and Bioproducts Processing, 91 (2013) 303-309) se estudió la extracción PLE de cafeína de granos de café verde, enteros y triturados, utilizando como disolventes lactato de etilo, acetato de etilo y etanol, a la temperatura de 100, 150 y 200 ºC.

En general, para los tres disolventes estudiados, la mayor concentración de cafeína en los extractos se obtuvo a 150 ºC, indicando un posible máximo de selectividad alrededor de esta temperatura. No obstante, a 200 ºC (máxima temperatura de extracción estudiada) esta selectividad no disminuyó de forma excesiva, mientras que la cantidad total de cafeína extraída aumentó notablemente debido a los altos rendimientos de extracción (masa total de sólidos extraída) que se obtuvieron a esta temperatura.

La Tabla 4.2 muestra la recuperación de cafeína (cafeína extraída respecto al contenido inicial de cafeína presente en los granos) obtenida con cada uno de los disolventes y su concentración en el extracto a 200 ºC. Igualmente, se muestra la concentración y recuperación de compuestos fenólicos y lípidos obtenida. Como puede comprobarse, con acetato de etilo, uno de los disolventes actualmente utilizados a nivel comercial en el descafeinado de café, se obtuvieron en el extracto concentraciones de cafeína considerablemente mayores que con lactato de etilo o etanol, tanto a partir de los granos enteros, como triturados. Por lo tanto, el acetato de etilo demostró ser el

disolvente más selectivo en la extracción de cafeína de granos de café verde. Sin embargo, la recuperación obtenida con acetato de etilo fue la menor, por lo que sería necesario un mayor consumo de disolvente para conseguir un grado de descafeinado similar al obtenido con los otros dos disolventes estudiados. Por el contrario, el lactato de etilo fue el disolvente con el que se obtuvo la mayor recuperación de cafeína. A 200 ºC, estas recuperaciones fueron un 23 % y un 28 % más altas que las obtenidas con acetato de etilo.

En la Tabla 4.2 también puede observarse que la extracción de compuestos de naturaleza lipídica fue similar con lactato de etilo y acetato de etilo. Esto es muy importante desde un punto de vista de la calidad del producto descafeinado, ya que estos compuestos, junto con los compuestos fenólicos, participan en las características sensoriales del café, las cuales se desarrollan principalmente durante la etapa de tostado, posterior al descafeinado de los granos de café verde. Por otro lado, pese a que el lactato de etilo no resultó ser peor que los otros disolventes estudiados respecto a la co-extracción de compuestos de naturaleza lipídica, en el caso de los compuestos fenólicos, las cantidades extraídas son superiores a las obtenidas con acetato de etilo.

Tabla 4.2. PLE de granos de café verde, a 200 ºC. Concentración (C) (mg / g de extracto) y recuperación (R %) de cafeína, compuestos fenólicos y compuestos lipídicos, obtenidos con lactato de etilo, etanol y acetato de etilo.

	Cafeína		Compuestos fenólicos		Compuestos lipídicos	
	C	R %	C	R %	C	R %
Lactato de etilo						
Grano entero	43,0	57,6	86,7	23,7	46,6	5,5
Grano triturado	58,3	63,1	126,7	27,8	104,0	9,7
Etanol						
Grano entero	78,1	47,3	139,4	17,1	65,9	3,5
Grano triturado	67,8	63,2	139,1	26,2	255,6	20,6
Acetato de etilo						
Grano entero	117,5	41,2	174,4	12,3	136,0	4,1
Grano triturado	101,5	48,8	157,4	15,3	289,8	12,0

En relación al etanol (Tabla 4.2.), se obtuvo una recuperación de cafeína prácticamente idéntica a la del lactato de etilo cuando la extracción se llevó a cabo con

granos de café triturados. No obstante, la concentración en el extracto y el rendimiento de extracción de compuestos lipídicos fue considerablemente mayor que el obtenido con lactato de etilo. En este sentido, el lactato de etilo podría ser interesante en la producción de café soluble descafeinado, en la cual podrían utilizarse granos de café triturados, aplicando una primera etapa de extracción con lactato de etilo para la eliminación de cafeína, seguida de la extracción con agua para producir el extracto de café soluble descafeinado.

4.2.2. Extracción de cafeína de hojas de té verde.

En el trabajo titulado "*Pressurized liquid extraction of caffeine and catechins from green tea leaves using ethyl lactate, water and ethyl lactate + water mixtures*", se estudió la extracción PLE de cafeína de hojas de té verde, utilizando como disolventes lactato de etilo, agua y distintas mezclas lactato de etilo / agua.

En la última década, el consumo de té ha aumentado considerablemente en occidente, debido en parte a sus propiedades beneficiosas para la salud, muchas de ellas atribuidas a los compuestos fenólicos del té y, especialmente, a las catequinas. La pérdida de compuestos fenólicos en el té verde, no sólo altera las características sensoriales del producto sino que también produce una reducción de sus propiedades funcionales, por lo que es importante preservar estos compuestos en la matriz vegetal durante el descafeinado. No obstante, la eliminación de cafeína, alterando mínimamente el contenido inicial de catequinas, es un proceso difícil de lograr. Actualmente, existen numerosos trabajos acerca de la extracción de cafeína y catequinas mediante diferentes técnicas y con distintos disolventes, así como procedimientos para su posterior separación y purificación.

Nuevamente, el acetato de etilo es uno de los disolventes principalmente utilizados a nivel comercial en la producción de té descafeinado debido a su alta selectividad por la cafeína, pero la pérdida de catequinas presentes en la hoja de té verde es considerable. De hecho, el acetato de etilo ha sido estudiado en procesos de extracción de catequinas (Piñeiro y col., 2004) y en procesos de purificación de catequinas a partir de extractos acuosos de té verde (Row y Jin, 2006), obteniéndose muy buenos resultados. El disolvente cloruro de metileno ha demostrado ser un disolvente muy eficiente en la extracción de cafeína, alterando mínimamente el contenido de catequinas (Choung y col., 2014). No obstante, como se ha comentado a lo largo de la presente memoria, su

uso tiende a eliminarse debido a su toxicidad y a los problemas medioambientales que genera. Por ello, la búsqueda de nuevos disolventes agroquímicos, ecológicos y no tóxicos, así como nuevos procedimientos de extracción, que permitan la obtención de hojas de té verde con un bajo contenido en cafeína, constituye un interesante campo de investigación.

La Tabla 4.3 muestra la recuperación de cafeína y catequinas obtenida en la extracción de hojas de té verde con lactato de etilo y agua presurizados, entre 50 y 200 ºC. Con lactato de etilo se alcanzó una extracción de aproximadamente el 92 % de la cafeína presente en el té, tras 20 minutos de extracción a 200 ºC. Sin embargo, a temperaturas superiores a 150 ºC, se observó una clara disminución de la recuperación de catequinas, probablemente como consecuencia de su degradación térmica, especialmente acusada en el caso del agua como disolvente de extracción. Aunque con lactato de etilo también se observó cierta degradación, ésta fue mucho menor que la observada con agua.

Tabla 4.3. PLE de té verde. Recuperación de cafeína y catequinas obtenida con lactato de etilo y agua, a temperaturas de extracción entre 50 ºC y 200 ºC.

	Recuperación (%)			
	50 ºC	100 ºC	150 ºC	200 ºC
Lactato de etilo				
Cafeína	10,7	50,0	75,9	91,7
Catequinas	7,6	26,0	36,3	28,6
Agua				
Cafeína	42,0	53,3	76,3	76,2
Catequinas	40,5	43,1	44,7	13,9

La Tabla 4.4 muestra los resultados obtenidos a 100 ºC, utilizando como disolventes lactato de etilo, agua y la mezcla lactato de etilo / agua (50:50). La tabla incluye la selectividad cafeína / catequinas, calculada según la ecuación:

$$S = k_1/k_2$$

donde k_1 y k_2 son, respectivamente, el coeficiente de distribución de cafeína y catequinas.

Como puede observarse en la tabla, la mayor selectividad cafeína / catequinas se obtuvo con lactato de etilo. Con la mezcla lactato de etilo / agua (50:50) la recuperación de cafeína y catequinas fue más elevada que con lactato de etilo puro, pero la selectividad del procesos de separación no es mejor que con lactato de etilo puro. Para determinar alguna ventaja al utilizar la mezcla lactato de etilo / agua, sería importante evaluar las características organolépticas del producto descafeinado que se obtiene utilizando uno u otro disolvente.

Tabla 4.4. PLE de té verde, a 100 ºC. Concentración (C) (mg / g de extracto), recuperación (R) de cafeína y catequinas, y selectividad cafeína / catequinas (S).

	Cafeína		Catequinas		S
	C	R (%)	C	R (%)	
Lactato de etilo	125,1	50,0	204,8	26,0	2,8
Agua	48,0	53,3	122,2	43,1	1,5
Lactato de etilo / Agua (50:50)	50,9	74,8	109,7	51,2	2,8

Por lo tanto, el lactato de etilo podría ser un disolvente alternativo para la extracción sólido-líquido de cafeína de materias primas de origen vegetal, superior a otros disolventes verdes como agua o etanol y similar al acetato de etilo en cuanto a su selectividad, con la ventaja de su origen agroquímico. Por ejemplo, la selectividad cafeína / compuestos fenólicos y cafeína / compuestos lipídicos que presentó el acetato de etilo en la extracción de granos de café verde enteros a 200 ºC fue, respectivamente, 5,0 y 16,6. La selectividad cafeína / compuestos fenólicos del lactato de etilo fue similar (4,6) y en el caso de cafeína / compuestos lípidos fue mayor (25,1) a la obtenida con acetato de etilo a las mismas condiciones de extracción. No obstante, serían necesarios más estudios para determinar el efecto de la extracción de compuestos distintos a la cafeína en las características sensoriales del producto final.

Por otra parte, la tecnología de extracción con líquidos presurizados constituye una alternativa a los métodos de extracción sólido-líquido tradicionales, ya que pueden obtenerse altos rendimientos de extracción de cafeína en cortos periodos de tiempo. Como ejemplo, podemos mencionar el estudio realizado por Perva-Uzunalic y col. (2006), en el que tras 120 minutos de extracción por reflujo de hojas de té verde utilizando distintos disolventes orgánicos y sus mezclas con agua, se alcanzaron

cantidades extraídas de cafeína similares o menores a las obtenidas en la presente memoria utilizando tan sólo 20 minutos de extracción.

4.2.3. Extracción supercrítica de cafeína de hojas de té verde utilizando lactato de etilo como modificador

La tecnología de extracción con fluidos supercríticos, principalmente utilizando CO_2 supercrítico, constituye el otro procedimiento general de descafeinado de matrices vegetales a nivel industrial. Si bien la selectividad del CO_2 supercrítico por la cafeína es alta, la solubilidad de cafeína en CO_2 es baja, por lo que se requiere el uso de modificadores para que el proceso pueda llevarse a cabo de una manera eficiente. El agua es el modificador utilizado a nivel industrial en la producción de granos de café y hojas de té verde descafeinadas, para aumentar la disponibilidad de la cafeína contenida en la matriz vegetal y para aumentar su difusión. En el caso de la extracción de cafeína de hojas de té verde, los trabajos presentados en la bibliografía hasta el momento solo se han referido al estudio del agua y etanol como cosolventes.

En el trabajo titulado "*Effect of cosolvents (ethyl lactate, ethyl acetate and ethanol) on the supercritical CO_2 extraction of caffeine from green tea*" (The Journal of Supercritical Fluids, en prensa), se estudió la eficacia de tres cosolventes: etanol, acetato de etilo y lactato de etilo, en la extracción de cafeína de té verde. En este estudio, la mayor cantidad de cafeína se obtuvo con lactato de etilo, independientemente del mecanismo de extracción, estática o dinámica, con un rendimiento de extracción de cafeína de 13,0 mg/g y 14,2 mg/g, respectivamente. En el caso del etanol, este rendimiento de extracción fue un 17 % (extracción estática) y un 38 % (extracción dinámica) menor que el obtenido con lactato de etilo, mientras que el rendimiento obtenido con acetato de etilo fue el más bajo (un 49 % y un 71 % menor) y muy similar al obtenido con CO_2 sin la adición de cosolventes. Además, con lactato de etilo la velocidad de extracción de cafeína en los primeros instantes de la extracción, en la que el soluto se encuentra fácilmente accesible, fue un 40 % y un 80 % mayor que la velocidad de extracción con etanol y acetato de etilo, respectivamente. Este comportamiento podría deberse a una mayor solubilidad de la cafeína en la fase supercrítica CO_2 + lactato de etilo. No obstante, no hay datos disponibles en la bibliografía para comprobarlo.

Considerando los trabajos publicados, Park y col., (2007[b]) estudiaron la extracción de cafeína de hojas de té verde a 30 MPa y 70 ºC, utilizando agua como cosolvente. Los autores obtuvieron, a las mismas condiciones de presión y temperatura, una recuperación de cafeína del 77 %, superior a la obtenida en esta tesis con lactato de etilo (48 % y 53 % en la extracción estática y dinámica, respectivamente). Sin embargo, debe tenerse en cuenta que Park y col. utilizaron un porcentaje de cosolvente considerablemente mayor (8,8 % frente al 2,2 % en peso) y relaciones CO_2 / té verde unas 2 veces superiores a las utilizadas en esta tesis. Con un 5,8 % de cosolvente, la recuperación obtenida por Park y col., (2007[b]) fue 42 %, inferior a la obtenida con lactato de etilo, pese a seguir utilizando un porcentaje de cosolvente y una relación CO_2 / té verde mucho mayor a los utilizados en esta tesis. En este sentido, es importante investigar el proceso de descafeinado supercrítico con lactato de etilo como modificador con el objetivo de optimizar el porcentaje de modificador y la relación CO_2 / té. Los primeros resultados obtenidos en esta tesis parecen indicar que la extracción supercrítica con lactato de etilo como modificador podría implicar una disminución en la relación CO_2 / té verde y/o una reducción del tiempo de extracción necesario para llevar a cabo el proceso descafeinado.

4.3. Producción de un extracto concentrado en catequinas y bajo en cafeína a partir de té verde.

En la extracción de cafeína de té verde mediante líquidos presurizados, a pesar de la mayor selectividad cafeína / catequinas del lactato de etilo, se produjo una pérdida de catequinas. Debido a la buena selectividad del lactato de etilo por la cafeína, y al análisis de los datos de equilibrio del sistema binario CO_2 + lactato de etilo, que indicaron la formación de una fase homogénea a presiones mayores de 10 MPa y temperaturas moderadas, se planteó la posibilidad de recuperar dichas catequinas para obtener un extracto seco con bajo contenido en cafeína, mediante la tecnología verde SAS, que pudiera ser utilizado como un ingrediente funcional.

En el trabajo titulado "*High catechins/low caffeine powder from green tea leaves by pressurized liquid extraction and supercritical antisolvent precipitation*" (Separation and Purification Technology, 148 (2015) 49-56) se llevó a cabo el estudio de la precipitación solvente antisolvente (SAS) con CO_2 supercrítico, de los extractos PLE de hojas de té verde obtenidos con lactato de etilo, para producir un extracto rico en

catequinas y bajo en cafeína. Además, el mismo proceso se llevó a cabo con etanol, con el fin de comparar ambos disolventes agroquímicos.

Las extracciones PLE se llevaron a cabo a 100 ºC, por ser la temperatura a la que se obtuvo el extracto de lactato de etilo con mayor concentración de catequinas y al considerar que a esta temperatura la degradación térmica de las catequinas es baja. Respecto al proceso SAS, el flujo y las condiciones de presión más bajas a las que se llevó a cabo el proceso fueron las mínimas necesarias para producir un extracto libre de disolvente a las temperaturas de precipitación estudiadas.

Con lactato de etilo se obtuvieron precipitados con concentraciones de cafeína menores del 1 % y con concentraciones de catequinas alrededor de 1,4 veces mayores que las presentes en el extracto PLE de partida. Aunque el extracto PLE etanólico presentó una concentración de cafeína menor y una concentración de catequinas mayor que el extracto PLE obtenido con lactato de etilo, los precipitados del extracto PLE etanólico presentaron concentraciones de cafeína superiores al 2 % y concentraciones de catequinas muy similares a las del extracto de partida, no observándose un efecto de concentración de esas catequinas en el precipitado. Además, con etanol, los rendimientos de precipitación fueron inferiores a los obtenidos a partir de los extractos con lactato de etilo.

En Europa, el Comité Europeo del Té sugiere que el contenido máximo de cafeína de un extracto, para ser considerado descafeinado, deba ser como máximo 1,2 %. Combinando la extracción con lactato de etilo presurizado y la precipitación solvente-antisolvente del extracto con CO_2 supercrítico, se obtuvieron precipitados prácticamente exentos de cafeína, que pueden considerarse descafeinados, y con altas concentraciones de compuestos fenólicos y, en particular, de catequinas. Además, el proceso se llevó a cabo en un corto período de tiempo, a diferencia de la separación con membranas y con adsorbentes (Li y col., 2005), y utilizando un disolvente verde como es el lactato de etilo y dos técnicas respetuosas con el medio ambiente, como son, la extracción con líquidos presurizados y la precipitación solvente-antisolvente con CO_2 supercrítico, a diferencia de los procesos de extracción líquido-líquido con disolventes clorados (Row y Jin, 2006). A esto hay que añadir que el producto obtenido se obtuvo libre de disolvente, por lo que no sería necesaria una etapa posterior para su eliminación, a diferencia de los otros procesos de separación.

El extracto seco obtenido podría ser utilizado como nutracéutico o como un extracto de té verde funcional rico en catequinas y libre de cafeína, que podría ser producido a partir de subproductos de la industria del té o a partir del residuo procedente del descafeinado del té verde, para recuperar así la cafeína y las catequinas, ambos de interés en la industria.

5

CONCLUSIONES

5. CONCLUSIONES

Las conclusiones que derivan de esta tesis se pueden dividir en 3 apartados, en coincidencia con las tres etapas definidas en el plan de trabajo:

- **Medición de la solubilidad y del equilibrio de fases de mezclas que contienen lactato de etilo**:
  - La solubilidad de cafeína en lactato de etilo, a presión ambiente y a las condiciones de temperatura estudiadas (25-50 °C), fue entre 1,3 y 3,6 veces mayor que la solubilidad descrita en otros disolventes orgánicos, tales como metanol, acetona, etanol o acetato de etilo.
  - A pesar de que la solubilidad de cafeína en lactato de etilo fue similar e incluso menor que en agua, la presencia de agua en lactato de etilo produjo un efecto cosolvente que originó un considerable aumento de la solubilidad de algunas de las sustancias estudiadas, entre las que se encuentra la cafeína.
  - A bajas presiones (10 MPa) y a las temperaturas habitualmente aplicadas en extracción supercrítica, el dióxido de carbono y el lactato de etilo forman una fase homogénea, con potencial aplicación en tecnología supercrítica de forma similar a como se utiliza el etanol.

- **Extracción de cafeína a partir de granos de café verde y hojas de té verde utilizando lactato de etilo y otros disolventes verdes**:
  - En la extracción PLE de los granos de café verde enteros, la recuperación de cafeína fue mayor a la de los otros disolventes estudiados (etanol y acetato de etilo) al aplicar temperaturas de 150-200 °C. A 200 °C y con 20 minutos de extracción, se recuperó casi un 60% de la cafeína presente originalmente en los granos de café. Además, en ese rango de temperaturas, se observó una baja coextracción de compuestos lipídicos y fenólicos, similar a la de los otros disolventes estudiados (recuperaciones menores a 6 % y 24 % respectivamente).
  - En la extracción PLE de hojas de té verde, en el rango de 150-200 °C, la recuperación de cafeína con lactato de etilo fue similar a la obtenida utilizando etanol y mayor que la obtenida con agua. Con 20 minutos de extracción fue posible extraer entre el 76-92 % de la cafeína presente originalmente en las

hojas de té. Además, se determinó una menor degradación de catequinas cuando se utilizó lactato de etilo como disolvente, y una mayor selectividad cafeína / catequinas.

- En la extracción PLE de hojas de té verde utilizando mezclas (lactato de etilo + agua), aunque se produjo un aumento considerable de la recuperación de cafeína, permitiendo así la extracción a menor temperatura, no se obtuvo una selectividad cafeína / catequinas mejor que en el caso de utilizar lactato de etilo puro. Particularmente, la mezcla 50:50 en volumen, mostró un comportamiento intermedio entre el agua y el lactato de etilo, obteniéndose recuperaciones más parecidas a las del agua pura y una selectividad similar a la del lactato de etilo puro a la misma temperatura.
- En la extracción SFE de cafeína de hojas de té verde, el efecto de los distintos cosolventes utilizados fue: lactato de etilo > etanol > acetato de etilo. Es decir, utilizando lactato de etilo como cosolvente del CO_2, se obtuvo la mayor recuperación de cafeína y el mayor coeficiente de transferencia de materia, con recuperaciones 2 veces mayores que con CO_2 puro, tanto en los ensayos de extracción dinámica como estática.

- **Obtención de un producto concentrado en catequinas y bajo en cafeína, a partir de un extracto de té verde**:
  - El proceso solvente-antisolvente (SAS) de la tecnología de fluidos supercríticos, aplicado a los extractos PLE de té verde obtenidos con lactato de etilo, resultó en rendimientos de precipitación más altos, precipitados con menor contenido de cafeína y mayor relación catequinas / cafeína, que los obtenidos a partir de los extractos PLE con etanol, a pesar que los extractos etanólicos contenían menor concentración de cafeína y mayor concentración de catequinas.
  - Mediante un procedimiento combinado, totalmente verde, de extracción PLE con lactato de etilo y de precipitación SAS, se obtuvo un producto seco con un 23 % de catequinas y menos de 1 % de cafeína (% m/m), que podría ser utilizado como ingrediente funcional en la elaboración de productos derivados de té verde que no contengan cafeína.

Por lo tanto, la aplicación de lactato de etilo en procesos de extracción y/o fraccionamiento de cafeína de matrices vegetales, para la producción de alimentos o ingredientes descafeinados, es un procedimiento viable para la industria alimentaria, reemplazando con mayor selectividad y eficacia a otros disolventes actualmente utilizados para este fin.

Por otro lado, con la tecnología supercrítica, a través de la precipitación solvente-antisolvente, es posible separar con gran eficacia cafeína de catequinas utilizando sólo disolventes verdes, para obtener extractos de té verde descafeinados y concentrados en catequinas.

5. CONCLUSIONS

Conclusions derived from this PhD Dissertation can be classified considering the 3 stages defined in the work plan.

- **Solubility and phase equilibria measurements of mixtures containing ethyl lactate:**
  - The solubility of caffeine in ethyl lactate at ambient pressure and at the temperature conditions studied (25-50 °C) was from 1.3 to 3.6 times higher than the solubility reported for other organic solvents, such as methanol, acetone, ethanol or ethyl acetate.
  - Even though the solubility of caffeine in ethyl lactate was similar and even lower than in water, the presence of water added to ethyl lactate generated a cosolvent effect producing a solubility increase for some of the studied substances, caffeine among them.
  - Carbon dioxide and ethyl lactate forms a uniform phase at low pressures (10 MPa) and temperatures usually applied for supercritical extraction, with potential application in supercritical technology, similarly to the use of ethanol.

- **Extraction of caffeine from green coffee beans and green tea leaves using ethyl lactate and other green solvents**:
  - In the PLE extraction of the entire green coffee beans, the recovery of caffeine applying temperatures from 150-200 °C was higher than the one obtained with the other solvents studied (ethanol and ethyl acetate). At 200 °C and 20 minutes of extraction time, the recovered caffeine was almost 60 % of the initial caffeine contained in the coffee beans. Moreover, a low co-extraction of lipid-type compounds and phenolic compounds, which was similar to the obtained with the other studied green solvents, was observed at tested range of temperatures (recoveries lower than 6 % and 24 %, respectively).
  - In the PLE extraction of green tea leaves, the recovery of caffeine using ethyl lactate was similar to that obtained with ethanol, and higher than the one obtained with water, in the range of 150-200 °C. After 20 minutes of

extraction it was possible to extract between 76-92 % of the caffeine initially contained in the tea leaves. Additionally, a lesser degradation of catechins and higher caffeine / catechis selectivity was determined when ethyl lactate was used as a solvent.

- In the PLE extraction of green tea leaves using (ethyl lactate + water) mixtures, although an appreciable increase of the recovery of caffeine was observed, thus allowing the extraction at lower temperature, a better caffeine /catechins selectivity compared to the use of pure ethyl lactate was not achieved. Particularly, the mixture 50:50 volume showed a midway behavior between the water and the ethyl lactate, reaching more similar recoveries than those of pure ethyl lactate at the same temperature.
- In the SFE extraction of caffeine from green tea leaves, the effect of the different cosolvents used was: ethyl lactate > ethanol > ethyl acetate. That is, the highest recovery of caffeine and the highest mass transfer coefficient was achieved using ethyl lactate as a cosolvent of CO_2, reaching recoveries 2 times higher than with pure CO_2, in both dynamic extraction and static extraction assays.

- **Obtaining a product with high concentrations of catechins and low content of caffeine, from a green tea extract**:
  - As a result of the Supercritical Antisolvent process (SAS) applied to the PLE extracts of green tea obtained with ethyl lactate, higher precipitation yields, precipitates with a lower content of caffeine and larger catechins / caffeine ratio than the those achieved from the PLE extracts of ethanol were obtained, despite the ethanolic extracts contained lower concentration of caffeine and higher concentration of catechins.
  - By means of a complete environmentally friendly combined procedure consisting of a PLE extraction using ethyl lactate and SAS precipitation procedure, a dry product containing 23 % of catechins and less than 1 % of caffeine (% m/m) was achieved, which could be used as a functional ingredient in the production of green tea derived caffeine-free products.

Therefore, the application of ethyl lactate in caffeine extraction and/or fractionation processes from vegetable matrices for the production of decaffeinated foods or

ingredients, constitute a viable procedure for the food industry, replacing with greater selectivity and efficacy other solvents presently used.

Additionally, using the supercritical technology, by means of the antisolvent precipitation, it is possible to separate efficiently caffeine from catechins using only green solvents, to obtain decaffeinated green tea extracts containing high concentration of catechins.

6

BIBLIOGRAFÍA

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Alonso-Salces, R. M., Serra, F., Reniero, F., Heberger, K. *Botanical and geographical characterization of green coffee (Coffea arabica and Coffea canephora): chemometric evaluation of phenolic and methylxanthine contents*. Journal of Agricultural and Food Chemistry 57 (2009) 4224-4235.

Anastas, P. T., Nicolas, E. *Green Chemistry: principles and practice*. Chemical Society Reviews 39 (2010) 301-312.

Anastas, P. T., Kirchhoff, M. M. *Origins, current status, and future challenges of green chemistry*. Accounts of Chemical Research 35 (2002) 686-694.

Anastas, P. T., Warner, J. C. (1998). Green Chemistry: Theory and Practice. New York, USA: Oxford University Press.

Aparicio, S., Alcalde, R. *The green solvent ethyl lactate: an experimental and theoretical characterization*. Green Chemistry 11 (2009) 65-78.

Araújo, J. M. A., Sandi, D. *Extraction of coffee diterpenes and coffee oil using supercritical carbon dioxide*. Food Chemistry 101 (2006) 1087-1094.

Azevedo, A. B. A., Mazzafera, P., Mohamed, R. S., Vieira de Melo, S. A. B. Kieckbusch, T. G. *Extraction of caffeine, chlorogenic acids and lipids from green coffee beans using supercritical carbon dioxide and co-solvents*. Brazilian Journal of Chemical Engineering 25 (2008) 543-552.

Bansal, S., Syan, N., Mathur, P., Choudhary, S. *Pharmacological profile of green tea and its polyphenols: a review*. Medicinal Chemistry Research 21 (2012) 3347-3360.

Brunner, G. *Supercritical fluids: technology and application to food processing*. Journal of Food Engineering 67 (2005) 21-33.

Budryn, G., Nebesny, E., Podsedek, A., Zyzelewicz, D., Materska, M., Jankowski, S., Janda, B. *Effect of different extraction methods on the recovery of chlorogenic acids, caffeine and Maillard reaction products in coffee beans*. European Food Research and Technology 228 (2009) 913-922.

Carabias-Martínez, R., Rodríguez-Gonzalo, E., Revilla-Ruiz, P., Hernández-Méndez, J. Pressurized liquid extraction in the analysis of food and biological samples. Journal of Chromatography A 1089 (2005) 1-17.

Carvalho, R. N. J., Moura, L. S., Rosa, P. T. V., Meireles, M. A. A. *Supercritical fluid extraction from rosemary (Rosmarinus officinalis): kinetic data, extract's global yield, composition, and antioxidant activity*. Journal of Supercrital Fluids 35 (2005) 197-204.

Chaturvedula, V. S. P., Prakash, I. *The aroma, taste, color and bioactive constituents of tea*. Journal of Medicinal Plants Research 5 (2011) 2110-2124.

Chemat, F., Vian, M. A., Cravotto, G. *Green extraction of natural products: concept and principles*. International Journal of Molecular Sciences 13 (2012) 8615-8627.

Chen, D., Wan, S. B., Yang, H., Yuan, J., Chan, T. H., Dou, Q. P. *EGCG, green tea polyphenols and their synthetic analogs and prodrugs for human cancer prevention and treatment*. Advances in Clinical Chemistry 53 (2011) 155-177.

Chen, Z. Y., Zhu, Q. Y., Wong, Y. F., Zhang, Z., Chung, H. Y. *Stabilizing effect of ascorbic acid on green tea catechins*. Journal of Agricultural and Food Chemistry 46 (1998) 2512-2516.

Chimowitz, E. H., Pennisi, K. J. *Process synthesis concepts for supercritical gas extraction in the crossover region*. AIChE Journal 32 (1986) 1665-1676.

Choung, M. G., Hwang, Y. S., Lee, M. S., Lee, J., Kang, S. T., Jun, T. H. *Comparison of extraction and isolation efficiency of catechins and caffeine from green tea leaves using different solvent systems*. International Journal of Food Science and Techonlogy 49 (2014) 1572-1578.

Choung, M. G., Lee, M. S. *Optimal extraction conditions for simultaneous determination of catechins and caffeine in green tea leaves*. Food Science and Biotechnology 20 (2011) 327-333.

Chylinski, K., Gregorowicz, J. *Solubilities of (1-hexanol, or 1,2-hexanediol, or 2-hydroxypropanoic acid ethyl ester, or 2-hydroxyhexanoic acid ethyl ester) in supercritical CO_2*. Journal of Chemical Thermodynamics 30 (1998) 1131-1140.

[a]Clarke, R.J. Coffee. (2003). Green coffee. In: Trugo, L., Finglas, P. M. (Eds.). Encyclopedia of Food Sciences and Nutrition, 2nd Edition (pp. 1481-1487). London, UK: Elsevier Ltd.

[b]Clarke, R.J. (2003). Roast and ground. In: Trugo, L., Finglas, P. M. (Eds.). Encyclopedia of Food Sciences and Nutrition, 2nd Edition (pp. 1487-1493). London, UK: Elsevier Ltd.

[c]Clarke, R.J. (2003). Decaffeination. In: Trugo, L., Finglas, P. M. (Eds.). Encyclopedia of Food Sciences and Nutrition, 2nd Edition (pp. 1506-1511). London, UK: Elsevier Ltd.

Cocero, M. J., Martín, A., Mattea, F., Varona, S. *Encapsulation and co-precipitation processes with supercritical fluids: Fundamentals and applications*. Journal of Supercritical Fluids 47 (2009) 546-555.

Dandia, A., Jain, A. K., Laxkar, A. K. *Ethyl lactate as promising bio based green solvent for the synthesis of spiro-oxindole derivatives via 1,3-dipolar cycloaddition reaction*. Tetrahedron Letters 54 (2013) 3929-3932.

Darr, J. A., Poliakoff, M. *New directions in inorganic and metal-organic coordination chemistry in supercritical fluids*. Chemical Reviews 99 (1999) 495-541.

Debry, G. (1994). Caffeine. In: Debry, G. (Ed.). Coffee and Health (pp. 111-156). London, UK: John Libbey & Company Ltd.

Devasagayam, T. P. A., Kamat, J. P., Mohan, H., Kesavan, P. C. *Caffeine as an antioxidant: inhibition of lipid peroxidation induced by reactive oxygen species*. Biochimica et Biophysica Acta 1282 (1996) 63-70.

Diplock, A. T., Aggett, P. J., Ashwell, M., Bornet, F., Fern, E. B., Roberfroid, M. B. *Scientific concepts of functional foods in Europe: consensus document*. British Journal of Nutrition 81 (1999) S1–S27.

Dobbs, J. M., Wong, J. M., Lahiere, R. J., Johnston, K. P. *Modification of supercritical fluid phase behavior using polar cosolvents*. Industrial and Engineering Chemistry Research 26 (1987) 56-65.

[a]Dong, J. J., Ye, J. H., Lu, J. L., Zheng, X. Q., Liang, Y. R. *Isolation of antioxidant catechins from green tea and its decaffeination*. Food and Bioproducts Processing 89 (2011) 62-66.

[b]Dong, Z. B., Liang, Y. R., Fan, F. Y., Ye, J. H., Zheng, X. Q., Lu, J. L. *Adsorption behavior of the catechins and caffeine onto polyvinylpolypyrrolidone*. Journal of Agricultural and Food Chemistry 59 (2011) 4238-4247.

Dos Santos, C. A. F., Leitao, A. E., Pais, I. P., Lindon, F. C., Ramalho, J. C. *Perspectives on the potential impacts of climate changes on coffee plant and bean quality*. Emirates Journal of Food and Agriculture 27 (2015) 152-163.

Drapeau, J., Verdier, M., Touraud, D., Kröckel, U., Geier, M., Rose, A., Kunz, W. *Effective insect repellent formulation in both surfactantless and classical microemulsions with a long-lasting protection for human beings*. Chemistry and Biodiversity 6 (2009) 934-947.

Engelhardt. U. H. (2010). Chemistry of tea. In: Mander, L., Liu, H. B. (Eds.). Comprehensive Natural Products II: Chemistry and Biology, Volume 3 (pp. 999-1032). London, UK: Elsevier Applied Science Publishers Ltd.

Erba, D., Riso, P., Bordoni, A., Foti, P., Biagi, P. L., Testolin, G. *Effectiveness of moderate green tea consumption on antioxidative status and plasma lipid profile in humans*. Journal of Nutritional Biochemistry 16 (2005) 144-149.

Esquivel, P., Jiménez, V. M. *Functional properties of coffee and coffee by-products*. Food Research International 46 (2012) 488-495.

Evstigneev, M. P., Evstigneev, V. P., Hernandez Santiago, A. A., Davies, D. B. *Effect of a mixture of caffeine and nicotinamide on the solubility of vitamin (B_2) in aqueous solution*. European Journal of Pharmaceutical Sciences 28 (2006) 59-66.

Fan, F. Y., Gan, Q., Dong, Z. B., Song, K. J., Zheng, X. Q., Liang, Y. R., Lu, J. L. *Selective elution of tea catechins and caffeine from polyvinylpolypyrrolidone*. International Journal of Food Science and Technology 49 (2014) 1626-1634.

Farah, A. (2012). Coffee constituents. In: Chu, Y. F. (Ed.). Coffee: Emerging Health Effects and Disease Prevention, 1st edition (pp. 22-58). New York, USA: Wiley & Sons, Inc.

Farah, A., Marino Donangelo, C. *Phenolic compounds in coffee*. Brazilian Journal of Plant Physiology 18 (2006) 23-36.

Farah, A., de Paulis, T., Moreira, D. P., Trugo, L. C., Martin, P. R. *Chlorogenic acids and lactones in regular and water-decaffeinated arabica coffees*. Journal of Agricultural and Food Chemistry 54 (2006) 374-381.

Fernández-Maestre, R., Velasco, A. R., Hill, H. H. *Explaining the drift behavior of caffeine and glucosamine after addition of ethyl lactate in the buffer gas of an ion mobility spectrometer*. Bulletin of the Korean Chemical Society 35 (2014) 1023-1028.

Fornari, T., Vicente, G., Vázquez, E., García-Risco, M. R., Reglero, G. *Isolation of essential oil from different plants and herbs by supercritical fluid extraction*. Journal of Chromatography A 1250 (2012) 34-48.

Foster, N. R., Singh, H., Yun, S. L. J., Tomasko, D. L., Macnaughton, S. J. *Polar and nonpolar cosolvent effects on the solubility of cholesterol in supercritical fluids*. Industrial and Engineering Chemistry Research 32 (1993) 2849-2853.

Foster, N. R., Gurdial, G. S., Yun, S. L. J., Liong, K. K., Tilly, K. D., Ting, S. S. T., Singh, H., Lee, J. H. *Significance of the crossover pressure in solid-supercritical fluid phase equilibria*. Ind. Eng. Chem. Res. 30 (1991) 1955-1964.

Friedman, M. (2007). *Overview of antibacterial, antitoxin, antiviral, and antifungal activities of tea flavonoids and teas*. Molecular Nutrition & Food Research, 51, 116-134.

Gadkari, P. V., Balaraman, M. *Catechins: sources, extraction and encapsulation: a review*. Food and Bioproducts Processing 93 (2015) 122-138.

Gadkari, P. V., Kadimi, Balaraman, M. *Catechin concentrates of garden tea leaves (Camellia sinensis L.): extraction/isolation and evaluation of chemical composition*. Journal of the Science of Food and Agriculture 94 (2014) 2921-2928.

Gao, J., Zhao, X. M., Zhou, L. Y., Huang, Z. H. *Investigation of ethyl lactate reactive distillation process*. Chemical Engineering Research and Design 85 (2007) 525-529.

Ghosh, P. P., Paul, S., Das, A. R. *Light induced synthesis of symmetrical and unsymmetrical dihydropyridines in ethyl lactate–water under tunable conditions*. Tetrahedron Letters 54 (2013) 138-142.

Giannellia, M., Doyle, P., Roman, E., Pelerin, M., Hermon, C. (2003). *The effect of caffeine consumption and nausea on the risk of miscarriage*. Paediatric and Perinatal Epidemiology (2003) 17 316-323.

Glade, M. J. *Caffeine-not just a stimulant*. Nutrition 26 (2010) 932-938.

Golmakani, M., Mendiola, J. A., Rezaei, K., Ibáñez, E. *Expanded ethanol with CO2 and pressurized ethyl lactate to obtain fractions enriched in α-linolenic acid from Arthrospira platensis (Spirulina)*. The Journal of Supercritical Fluids 62 (2012) 109-115.

Gottesman, M. (1985). Decaffeination of green coffee with n-butyl acetate. US 4562083.

Greff, R. J. (2000). Vascular embolizing compositions comprising ethyl lactate and methods for their use. US 6051607.

Grethe, T. (1935). Method for producing coffee free from caffeine. US 2016634.

Griffiths, R. R., Evans, S. M., Heishman, S. J., Preston, K. L., Sannerud, C. A., Wolf, B., Woodson, P. P. *Low-dose caffeine physical dependence in humans*. Journal of Pharmacology and Experimental Therapeutics 255 (1990) 1123-1132.

Guo, H., Wang, W., Sun, Y., Li, H., Ai, F., Xie, L., Wang, X. *Ethyl lactate enhances ethylenediaminedisuccinic acid solution removal of copper from contaminated soils*. Journal of Hazardous Materials 174 (2010) 59-63.

Gupta, S. K. (2006). Topical delivery system for cosmetic and pharmaceutical agents. US 0110415.

Hamden, K., Bengara, A.,Amri, Z., Elfeki, A. *Experimental diabetes treated with trigonelline: effect on key enzymes related to diabetes and hypertension, β-cell and liver function*. Molecular and Cellular Biochemistry 381 (2013) 85-94.

Härdtner, C., Multhoff, G., Falk, W., Radons, J. *(-)-Epigallocatechin-3-gallate, a green tea-derived catechin, synergizes with celecoxib to inhibit IL-1-induced tumorigenic mediators by human pancreatic adenocarcinoma cells Colo357*. European Journal of Pharmacology 684 (2012) 36-43.

Hartley, T. R., Sung, B. H., Pincomb, G. A., Whitsett, T. L., Wilson, M. F., Lovallo, W. R. *Hypertension risk status and effect of caffeine on blood pressure*. Hypertension 36 (2000) 137-141.

Henneberry, M., Snively, J. A., Vasek, G. J., Datta, R. (2004). Biosolvent composition of lactate ester and D-limonene with improved cleaning and solvating properties. US 6797684.

Henning, S. M., Fajardo-Lira, C., Lee, H. W., Youssefian, A. A., Go, V. L. W., Heber, D. *Catechin content of 18 teas and a green tea extract supplement correlates with the antioxidant capacity*. Nutrition and Cancer 45 (2003) 226-235.

Hernández, E. J., Luna, P., Stateva, R. P., Najdanovic-Visak, V., Reglero, G., Fornari, T. *Liquid-liquid phase transition of mixtures comprising squalene, olive oil, and ethyl lactate: application to recover squalene from oil deodorizer distillates*. Journal of Chemistry and Engineering Data 56 (2011) 2148-2152.

Herrero, M., Castro-Puyana, M., Mendiola, J. A., Ibáñez, E. Compressed fluids for the extraction of bioactive compounds. Trends in Analytical Chemistry 43 (2013) 67-83.

Hossain, M. M., Chong, W. E. *A new approach for removal of caffeine from coffee using sunflower oil*. Proceedings of the World Congress on Engineering 3 (2011) 2676-2680.

Huang, K. J., Wu, J. J., Chiu, Y. H., Lai, C. Y., Chang, C. M. J. *Designed polar cosolvent-modified supercritical CO_2 removing caffeine from and retaining catechins in green tea powder using response surface methodology*. Journal of Agricultural and Food Chemistry 55 (2007) 9014-9020.

Ibáñez, E., Kubátová, A., Señoráns, F. J., Cavero, S., Reglero, G., Hawthorne, S. B. *Subcritical water extraction of antioxidant compounds from rosemary plants*. Journal of Agricultural and Food Chemistry 51 (2003) 375-382.

International Agency for Research on Cancer (IARC). (1991). Coffee. In: IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans, Volume 5: Coffee, Tea, Mate, Methylxanthines and Methylglyoxal (pp. 41-197). Lyon, France: IARC Press.

Ishida, B. K., Chapman, M. H. *Carotenoid extraction from plants using a novel, environmentally friendly solven*. Journal of Agriculture and Food Chemistry 57 (2009) 1051-1059.

Iwai, Y., Nagano, H., Lee, G. S., Uno, M., Arai, Y. *Measurement of entrainer effects of water and ethanol on solubility of caffeine in supercritical carbon dioxide by FT-IR spectroscopy*. Journal of Supercritical Fluids 38 (2006) 312-318.

Jafar, J. J., Budd, P. M., Hughes, R. *Enhancement of esterification reaction yield using zeolite A vapour permeation membrane*. Journal of Membrane Science 199 (2002) 117-123.

Judge, M. D., Aab, C. *Ethyl lactate as an environmentally friendly HPLC mobile-phase modifier in the analysis of acetaminophen, caffeine, and ASA*. Canadian Journal of Chemistry 91 (2013) 352-356.

Jun, X. *Caffeine extraction from green tea leaves assisted by high pressure processing*. Journal of Food Engineering 94 (2009) 105-109.

Jung, J., Perrut, M. *Particle design using supercritical fluids: literature and patent survey*. Journal of Supercritical Fluids 20 (2001) 179-219.

Kaleda, W. W., Saleeb, F. Z., Zeller, B. L. (1988). Coffee decaffeination with caffeic acid. US 4767634.

Kanda, H., Li, P., Makino, H. *Production of decaffeinated green tea leaves using liquefied dimethyl ether*. Food and Bioproducts Processing 91 (2013) 376-380.

Kazlas, P. T., Novak, R. D., Robey, R. J. (1994). Supercritical carbon dioxide decaffeination of acidified coffee. US 5288511.

Kawakatsu, T., Kobayashi, T., Sano, Y., Nakajima, M. *Clarification of green tea extract by microfiltration and ultrafiltration*. Bioscience, Biotechnology, and Biochemistry 59 (1995) 1016-1020.

Khan, N., Mukhtar, H. *Tea polyphenols for health promotion*. *Life Sciences* 81 (2007) 519-533.

Kim, W. J., Kim, J. D., Kim, J., Oh, S. G., Lee, Y. W. *Selective caffeine removal from green tea using supercritical carbon dioxide extraction*. Journal of Food Engineering 89 (2008) 303-309.

Kim, S., Park, J., Lee, L., Han, D. *Effect of pH on the green tea extraction.* Korean Journal of Food Science and Technology 31 (1999) 1024-1028.

Kleinwachter, M., Bytof, G., Selmar, D. (2014). Coffee beans and processing. In: Preedy, V. R. (Ed.). Coffee in Health and Disease Prevention (pp. 73-82). London, UK: Elsevier Ltd.

Knez, Z., Weidner, E. *Particles formation and particle design using supercritical fluids*. Current Opinion in Solid State and Materials Science 7 (2003) 353-361.

Kopcak, U., Mohamed, R. S. *Caffeine solubility in supercritical carbon dioxide/co-solvents mixture*. Journal of Supercritical Fluids 34 (2005) 209-214.

Kronschläger, M., Löfgren, S., Yu, Z., Talebizadeh, N., Varma, S. D., Söderberg, P. *Caffeine eye drops protect against UV-B cataract*. Experimental Eye Research 113 (2013) 26-31.

Kumar, V., Ravishankar, G. A. *Current trends in producing low levels of caffeine in coffee berry and processed coffee powder*. Food Reviews International 25 (2009) 175-197.

Kündig, F. (1924). Process for the production of coffee free from caffeine. US 1629512.

Kurzhals, H. A. (1980). Process for the decaffeination of raw coffee beans. US 4207352.

Kwak, H., Hwang, D. W., Hwang, Y. K., Chang, J. S. *Recovery of alkyl lactate from ammonium lactate by an advanced precipitation process*. Separation and Purification Technology 93 (2012) 25-32.

Lack, E., Gamse, T., Marr, R. (2001). Extraction from solids. In: Bertucco, A., Vetter, G. (Eds.). High Pressure Process Technology: Fundamentals and Applications, Industrial Chemistry Library, Volume 9 (pp. 378-395). Amsterdam, The Netherlands: Elsevier Applied Science Publishers Ltd.

Lee, L. S., Kim, S. H., Kim, Y. B., Kim, Y. C. *Quantitative analysis of major constituents in green tea with different plucking periods and their antioxidant activity*. Molecules 19 (2014) 9173-9186.

Lee, H., Woo, H. C., Cho, S. H., Jung, Y. C., Seong, E. J., Jung, Y. H., Jeong, S. K. (2006). Supercritical fluid extraction method of caffeine. KR 20060119032.

Li, P., Wang, Y., Ma, R., Zhang, X. *Separation of tea polyphenol from green tea leaves by a combined CATUFM-adsorption resin process*. Journal of Food Engineering 67 (2005) 253-260.

Liang, H., Liang, Y., Dong, J., Lu, J., Xu, H., Wang, H. *Decaffeination of fresh green tea leaf (Camellia sinensis) by hot water treatment*. Food Chemistry 101 (2007) 1451-1456.

Lores, M., Pájaro, M., Álvarez-Casas, M., Domínguez, J., García-Jares, C. *Use of ethyl lactate to extract bioactive compounds from Cytisus scoparius: Comparison of pressurized liquid extraction and medium scale ambient temperature systems*. Talanta 140 (2015) 134-142.

Lou, Z., Er, C., Li, J., Wang, H., Zhu, S., Sun, J. *Removal of caffeine from green tea by microwave-enhanced vacuum ice water extraction*. Analytica Chimica Acta 716 (2012) 49-53.

Lu, J. L., Wu, M. Y., Yang, X. L., Dong, Z. B., Ye, J. H., Borthakur, D., Sun, Q. L., Liang, Y. R. *Decaffeination of tea extracts by using poly(acrylamide-co-ethylene glycol dimethylacrylate) as adsorbent*. Journal of Food Engineering 97 (2010) 555-562.

Ludwig, I. A., Mena, P., Calani, L., Cid, C., Del Rio, D., Lean, M. E. J., Crozier, A. *Variations in caffeine and chlorogenic acid contents of coffees: what are we driniking?*. Food and Function 5 (2014) 1718-1726.

Ludwig, I. A., Sanchez, L., Caemmerer, B., Kroh, L. K., De Peña, M., Concepción, C. *Extraction of coffee antioxidants: impact of brewing time and method*. Food Research International 48 (2012) 57-64.

Lynge, E., Anttila, A., Hemminki, K. *Organic solvents and cancer*. Cancer, Causes Control 8 (1997) 406-419.

MacHugh, M. A., Krukonis, V. J. (1994). Introduction. In: MacHugh, M. A., Krukonis, V. J. (Eds.). Supercritical Fluid Extraction. Principles and Practice, 2nd edition (pp. 1-16). MA, USA: Butterworth-Heinemann Series in Chemical Engineering.

Major, B., Németh, G., Bélafi-Bakó, K., Gubicza, L. *Unique role of water content in enzymatic synthesis of ethyl lactate using ionic liquid as solvent*. Chemical Papers 64 (2010) 261-264.

Mandel, H. G. *Update on caffeine consumption, disposition and action*. Food and Chemical Toxicology 40 (2002) 1231-1234.

Martín, A., Cocero, M. J. *Precipitation processes with supercritical fluids: patents review*. Recent Patents on Engineering 2 (2008) 9-20.

McMillan, B., Riggs, D. R., Jackson, B. J., Cunningham, C., McFadden, D. W. *Dietary influence on pancreatic cancer growth by catechin and inositol hexaphosphate*. Journal of Surgical Research 141 (2007) 115-119.

Méndez Sevillano, D., van der Wielen, L. A. M., Hooshyar, N., Ottens, M. *Resin selection for the separation of caffeine from green tea catechins*. Foods and Bioproducts Processing 92 (2014) 192-198.

Miyagishima, A., Fujiki, S., Okimura, A., Arahata, S., Inagaki, S., Iwao, Y., Itai, S. *Novel decaffeination of green tea using a special picking method and shortening of the rolling process*. Food Chemistry 125 (2011) 878-883.

Miyashita, Y., Etoh, H. *Improvement of the bitterness and astringency of green tea by sub-critical water extraction*. Food Science and Technology Research 19 (2013) 471-478.

Monsanto, M., Hooshyar, N., Meuldijk, J., Zondervan, E. *Modeling and optimization of green tea precipitation for the recovery of catechins*. Separation and Purification Technology 129 (2014) 129-136.

Morishita, H., Ohnishi, M. (2001). Absorption, metabolism and biological activities of chlorogenic acids and related compounds. In: Rahman, A. (Ed.). Studies in Natural Products Chemistry, Volume 25 (pp. 919-953). New York, USA: Elsevier Inc.

Nehlig, A., Daval, J., Debry, G. *Caffeine and the central nervous system: mechanism of action, biochemical, metabolic and psychostimulant effects*. Brain Research Reviews 17 (1992) 139-170.

Nikles, S. M., Piao, M., Lane, A. M. Nikles, D. E. *Ethyl lactate: a green solvent for magnetic tapecoating*. Green Chemistry 3 (2001) 109-113.

Nwuha, V. *Novel studies on membrane extraction of bioactive components of green tea in organic solvents: part I*. Journal of Food Engineering 44 (2000) 233-238.

Oestreich-Janzen, S. (2010). Chemistry of coffee. In: Townsend, C. A., Ebizuka, Y. (Eds.). Comprehensive Natural Products II: Chemistry and Biology, Volume 1 (pp. 1481-1487). London, UK: Elsevier Ltd.

Osterburg, A., Gardner, J., Hyon, S. H., Neely, A., Babcock, G. *Highly antibiotic-resistant Acinetobacter baumannii clinical isolates are killed by the green tea polyphenol (–)-epigallocatechin-3-gallate (EGCG)*. Microbiology and Infection 15 (2009) 341-346.

Pan, X., Niu, G., Liu, H. *Microwave-assisted extraction of tea polyphenols and tea caffeine from green tea leaves*. Chemical Engineering and Processing 42 (2003) 129-133.

Park, H. S, Im, N. G., Kim, K. H. *Extraction behaviors of caffeine and chlorophylls in supercritical decaffeination of green tea leaves*. Food Science and Technology 45 (2012) 73-78.

[a]Park, H. S., Choi, H. K., Lee, S. J., Park, K. W., Choi, S. G., Kim, K. H. *Effect of mass transfer on the removal of caffeine from green tea by supercritical carbon dioxide*. Journal of Supercritical Fluids 42 (2007) 205-211.

[b]Park, H. S., Lee, H. J., Shin, M. H., Lee K. W., Lee H., Kim, Y. S., Kim, K. O., Kim K. H. *Effects of cosolvents on the decaffeination of green tea by supercritical carbon dioxide*. Food Chemistry 105 (2007) 1011-1017.

Paston, S. V., Tarasov, A. E. *Effect of caffeine on DNA conformational changes after in vitro γ-irradiation*. Journal of Structural Chemistry 52 (2011) 1209-1214.

Peeling, P., Dawson, B. *Influence of caffeine ingestion on perceived mood states, concentration, and arousal levels during a 75-min university lecture*. Advances in Physiology Education 31 (2007) 332-335.

Pereira, C. S. M., Rodrigues, A. E. (2014). Ethyl lactate main properties, production processes, and applications. In: Chemat, F., Vian, M. A. (Eds.). Alternative Solvents for Natural Products Extraction (pp. 107-125). Berlin, DE: Springer International Publishing.

Pereira, C. S. M., Silva, V. M. T. M., Rodrigues, A. E. *Ethyl lactate as solvent: properties, applications and production processes – a review*. Green Chemistry 13 (2011) 2658-2671.

Pereira, C. S. M., Silva, V. M. T. M., Pinho, S. P., Rodrigues, A. E. *Batch and continuous studies for ethyl lactate synthesis in a pervaporation membrane reactor*. Journal of Membrane Science 361 (2010) 43-55.

Pereira, C. G., Meireles, M. A. A. *Supercritical fluid extraction of bioactive compounds: fundamentals, applications and economic perspectives*. Food and Bioprocess Technology 3 (2010) 340-372.

Pereira, C. S. M.; Zabka, M., Silva, V. M. T. M., Rodrigues, A. E. *A novel process for the ethyl lactate synthesis in a simulated moving bed reactor (SMBR)*. Chemical Engineering Science 64 (2009) 3301-3310.

Perrut, M. *Supercritical fluid applications: industrial developments and economic issues*. Industrial and Engineering Chemistry Research 39 (2000) 4531-4535.

Persson, I. A. L. (2013). Tea flavanols: an overview. In: Preedy, V. R. (Ed.). Tea in Health and Disease Prevention (pp. 73-78). London, UK: Elsevier Applied Science Publishers Ltd.

Perva-Uzunalic, A., Skerget, M., Knez, Z., Weinreich, B., Otto, F., Grüner, S. *Extraction of active ingredients from green tea (Camellia sinensis): extraction efficiency of major catechins and caffeine*. Food Chemistry 96 (2006) 597-605.

Piñeiro, Z., Palma, M., Barroso, C. G. *Determination of catechins by means of extraction with pressurized liquids*. Journal of Chromatography A 1026 (2004) 19-23.

Ramalakshmi, K., Raghavan, B. *Caffeine in coffee: its removal. Why and how?*. Critical Reviews in Food Science and Nutrition 39 (1999) 441-456.

Ramarethinam, S., Rajalakshmi, N. *Caffeine in tea plants [Camellia sinensis (L) O. Kuntze]: in situ lowering by Bacillus licheniformis (Weigmann) Chester*. Indian Journal of Experimental Biology 42 (2004) 575-580.

Reverchon, E., Adami, R. *Nanomaterials and supercritical fluids*. Journal of Supercritical Fluids 37 (2006) 1-22.

Reverchon, E. *Fractional separation of SCF extracts from marjoram leaves: mass transfer and optimization*. Journal of Supercritical Fluids 5 (1992) 256-261.

Richter, B. E., Jones, B. A., Ezzell, J. L., Porter, N. L. Accelerated solvent extraction: a technique form sample preparation. Analytical Chemistry 68 (1996) 1033-1039.

Rodríguez-Meizoso, I., Plaza, M. (2015). Particle Formation of Food Ingredients by Supercritical Fluid Technology. In: Fornari, T., Stateva, R. P. (Eds.). High Pressure Fluid Technology for Green Food Processing (pp. 155-184). Berlin, DE: Springer International Publishing.

Row, K., Jin, Y. *Recovery of catechin compounds from Korean tea by solvent extraction*. Bioresource Technology 97 (2006) 790-793.

Rusak, G., Komes, D., Likic, S., Horzic, D., Kovac, M. *Phenolic content and antioxidative capacity of green and white tea extracts depending on extraction conditions and the solvent used*. Food Chemistry 110 (2008) 852-858.

Sakanaka, S. *A novel convenient process to obtain a raw decaffeinated tea polyphenol fraction using a lignocellulose column*. Journal of Agricultural and Food Chemistry 51 (2003) 3140-3143.

Saldaña, M. D. A. (1997). Extraction of caffeine, trigonelline and chlorogenic acids from Brazilian coffee beans using supercritical CO_2. M.Sc. Thesis, State University of Campinas, Campinas, Brazil (en Portugués).

Salerno, A., Domingo, C. *Low-temperature clean preparation of poly(lactic acid) foams by combining ethyl lactate and supercritical CO_2: correlation between processing and foam pore structure*. Polymer International 63 (2014) 1303-1310.

Senol, A., Aydin, A. *Solid-liquid extraction of caffeine from tea waste using battery type extractor: process optimization*. Journal of Food Engineering 75 (2006) 565-573.

Shahidi, F., Naczk, M. (2004). Phenolic compounds of beverages. In: Shahidi, F., Naczk, M. (Eds.). Phenolics in Food and Nutraceuticals (pp. 241-312). Boca Raton, USA: Elsevier Applied Science Publishers Inc.

Shalmashi, A., Golmohammad, F. *Solubility of caffeine in water, ethyl acetate, ethanol, carbon tetrachloride, methanol, chloroform, dichloromethane, and acetone between 298 and 323 K*. Latin American applied research 40 (2010) 283-285.

Sheldon, R. A. *Green solvents for sustainable organic synthesis: state of the art*. Green Chemistry 7 (2005) 267-278.

Shi, Q. Y., Schlegel, V. *Green tea as an agricultural based health promoting food: the past five to ten years*. Agriculture 2 (2012) 393-413.

Silva, V. M. T. M., Pereira, C. S. M., Rodrigues, A. E. *PermSMBR—a new hybrid technology: application on green solvent and biofuel production*. AIChE Journal 57 (2010) 1840-1851.

Song, J., Lee, K., Seong, B. *Antiviral effect of catechins in green tea on influenza virus*. Antiviral Research 68 (2005) 66-74.

Sosa, M. V., Rodríguez-Rojo, S., Mattea, F., Cismondi, M., Cocero, M. J. *Green tea encapsulation by means of high pressure antisolvent precipitation*. Journal of Supercritical Fluids 56 (2011) 304-311.

Stadler, R. H., Richoz, J., Turesky, R. J., Welti, D. H., Fay, L. B. *Oxidation of caffeine and related methylxanthines in ascorbate and polyphenol-driven fenton-type oxidations*. Free Radical Research 24 (1996) 225-240.

Strati, I. F., Oreopoulou, V. *Effect of extraction parameters on the carotenoid recovery from tomato waste*. International Journal of Food Science and Technology 46 (2011) 23-29.

Su, Y. L., Leung, L. K., Huang, Y., Chen, Z. Y. *Stability of tea theaflavins and catechins*. Food Chemistry 83 (2003) 189-195.

Suárez-Quiroz, M. L., Taillefer, W., López Méndez, E. M., González-Ríos, O., Villeneuve, P., Figueroa-Espinoza, M. C. *Antibacterial activity and antifungal and anti-mycotoxigenic activities against Aspergillus flavus and A. ochraceus of green coffee chlorogenic acids and dodecyl chlorogenates*. Journal of Food Safety 33 (2013) 360-368.

Sun, Q. L., Hua, S., Ye, J. H., Lu, J. L., Zheng, X. Q., Liang, Y. R. *Decaffeination of green tea by supercritical carbon dioxide*. Journal of Medicinal Plants Research 4 (2010) 1161-1168.

Tang, W. Q., Li, D. C., Lv, Y. X., Jiang, J. G. *Extraction and removal of caffeine from green tea by ultrasonic-enhanced supercritical fluid*. Journal of Food Science 75 (2010) 363-368.

Teo, C. C., Tan, S. N., Yong, J. W. H., Hew, C. S., Ong, E. S. Pressurized hot water extraction (PHWE). Journal of Chromatography A 1217 (2010) 2484-2494.

Tfelt-Hansen, P., Saxena, P. R., Dahlöf, C., Pascual, J., Lainez, M., Henry, P., Diener, H. C., Schoenen, J., Ferrari, M. D., Goadsby, P. J. *Ergotamine in the acute treatment of migraine. A review and European consensus*. Brain 123 (2000) 9-18.

Theissing, P., Saamer, P., Korner, J. (1992). Decaffeinating coffee and tea. US 5153015.

Tombokan, X. C., Aguda, R. M., Danehower, D. A., Kilpatrick, P. K., Carbonell, R. G. *Three-component phase behavior of the sclareol-ethyl lactate-carbon dioxide system for GAS application*. Journal of Supercritical Fluids 45 (2008) 146-155.

Toro, P., Quijano, M. (1988). Process for the decaffeination of green coffee. US 4728525.

Turner, C., Ibáñez, E. (2012). Pressurized hot water extraction and processing. In: Lebovka, N., Vorobiev, E., Chemat, F. (Eds.). Enhancing Extraction Processes in the Food Industry (pp. 223-254). Boca ratón, USA: CRC Press, Taylor & Francis Group.

United States Department of Agriculture (USDA). Coffee: world markets and trade. December 2014.

Vicente, G., García-Risco, M. R., Fornari, T., Reglero, G. *Supercritical fractionation of Rosemary extracts to improve the antioxidant activity*. Chemical Engineering Technology 35 (2012) 176-182.

Vicente, G., Paiva, A., Fornari, T., Najdanovic-Visak, V. *Liquid-liquid equilibria of mixtures containing ethyl lactate, tocopherol and olive oil*. Chemical Engineering Journal 172 (2011) 879-884.

Vignoli, J. A., Bassoli, D. G., Benassi, M. T. *Antioxidant activity, polyphenols, caffeine and melanoidins in soluble coffee: the influence of processing conditions and raw material*. Food Chemistry 124 (2011) 863-868.

Vuong, Q. V., Roach, P. D. *Caffeine in green tea: its removal and isolation*. Separation and Purification Reviews 43 (2014) 155-174.

[a]Vuong, Q. V., Golding, J. B., Stathopoulos, C. E., Roach, P. D. *Effects of aqueous brewing solution pH on the extraction of the major green tea constituents*. Food Research International 53 (2013) 713-719.

[b]Vuong, Q. V., Golding, J. B., Nguyen, M. H., Roach, P. D. *Preparation of decaffeinated and high caffeine powders from green tea*. Powder Technology 233 (2013) 169-175.

Wachira, F. N., Kamunya, S., Karoi, S., Chalo, R.,Maritim, T. (2013). The tea plants: botanical aspects. In: Preedy, V. R. (Ed.). Tea in Health and Disease Prevention (pp. 3-19). London, UK: Elsevier Applied Science Publishers Ltd.

Wan, J. P., Cao, S., Jing, Y. *Copper-catalyzed homo- and cross-coupling reactions of terminal alkynes in ethyl lactate*. Applied Organometallic Chemistry 28 (2014) 631-634.

Wan, X., Li, D., Zhang, Z. (2009). Green tea and black tea. Manufacturing and consumption. In: Ho, C. T., Lin, J. K., Shahidi, F. (Eds.). Tea and Tea Products. Chemistry and Health-Promoting Properties (pp. 1-8). Boca Raton, USA: Elsevier Applied Science Publishers Inc.

Wang, R., Zhang, Y., Lan, Q., Holford, T. R., Leaderer, B., Zahm, S. H., Boyle, P., Dosemeci, M., Rothman, N., Zhu, Y., Qin, Q., Zheng, T. *Occupational exposure to solvents and risk of non-hodgkin lymphoma in Connecticut women*. American Journal of Epidemiology 169 (2009) 176-185.

Wang, R., Zhou, W., Jiang, X. *Reaction kinetics of degradation and epimerization of epigallocatechin gallate (EGCG) in aqueous system over a wide temperature range*. Journal of Agricultural and Food Chemistry 56 (2008) 2694-2701.

Wang, H., Helliwell, K. *Epimerisation of catechins in green tea infusions*. Food Chemistry 70 (2000) 337-344.

Wee, Y. J., Kim, J. N., Ryu, H. W. *Biotechnological production of lactic acid and its recent applications*. Food Technology and Biotechnology 44 (2006) 163-172.

Wei, K., Wang, L., Zhou, J., He, W., Zeng, J., Jiang, Y., Cheng, H. *Catechin contents in tea (Camellia sinensis) as affected by cultivar and environment and their relation to chlorophyll contents*. Food Chemistry 125 (2011) 44-48.

Weidner, E. *High pressure micronization for food applications*. Journal of Supercritical Fluids 47 (2009) 556-565.

Wicki, M. A., Nielsen, K. E. (2005). Process for preparing monoesters. US 0143599.

Wu, W., Lu, M., Yu, L. *A new environmentally friendly method for astaxanthin extraction from Xanthophyllomyces dendrorhous*. European Food Research and Technology 232 (2011) 463-467.

Xi, J., He, L., Yan, L. *Kinetic modeling of pressure-assisted solvent extraction of polyphenols from green tea in comparison with the conventional extraction*. Food Chemistry 166 (2015) 287-291.

Xi, J., Yan, L., He, L. *Pressure-dependent kinetic modeling of solid–liquid extraction of the major green tea constituents*. Separation and Purification Technology 133 (2014) 155-159.

Yan, Y., Li, J., Han, J., Hou, N., Song, Y., Dong, L. *Chlorogenic acid enhances the effects of 5-fluorouracil in human hepatocellular carcinoma cells through the inhibition of extracellular signal-regulated kinases*. Anti-Cancer Drugs 26 (2015) 540-546.

Yang, C. S., Wang, H., Li, G. X., Yang, Z., Guan, F., Jin, H. *Cancer prevention by tea: evidence from laboratory studies*. Pharmacological Research 64 (2011) 113-122.

Yap, C. L., Gan, S., Ng, H. K. *Feasibility of treating aged polycyclic aromatic hydrocarbons (PAHs)-contaminated soils using ethyl lactate-based Fenton treatment via parametric and kinetic studies*. Environmental Science and Pollution Research 22 (2015) 329-342.

Ye, J. H., Wang, L. X., Chen, H., Dong, J. J., Lu, J. L., Zheng, X. Q., Wu, M. Y., Liang, Y. R. *Preparation of tea catechins using polyamide*. Journal of Bioscience and Bioengineering 111 (2011) 232-236.

Ye, J. H., Dong, J. J., Lu, J. L., Zheng, X. Q., Jin, J., Chen, H., Liang, Y. R. *Effect of graft copolymerization of fir sawdust lignocellulose with N-vinylpyrrolidone on adsorption capacity to tea catechins*. Carbohydrate Polymers 81 (2010) 441-447.

Ye, J. H., Jin, J., Liang, H. L., Lu, J. L., Du, Y. Y., Zheng, X. Q., Liang, Y. R. *Using tea stalk lignocellulose as and adsorbent for separating decaffeinated tea catechins*. Bioresource Technology 100 (2009) 622-628.

Ye, J. H., Liang, Y. R., Jin, J., Liang, H. L., Du, Y. Y., Lu, J. L., Ye, Q., Lin, C. *Preparation of partially decaffeinated instant green tea*. Journal of Agricultural and Food Chemistry 55 (2007) 3498-3502.

Yixin, Q. U., Shaojun, P., Shui, W., Zhiqiang, Z., Jidong, W. *Kinetic study of esterification of lactic acid with isobutanol and n-butanol catalyzed by ion-exchange resins*. Chinese Journal of Chemical Engineering 17 (2009) 773-780.

Zaveri, N. T. *Green tea and its polyphenolic catechins: medicinal uses in cancer and noncancer applications*. Life Sciences 78 (2006) 2073-2080.

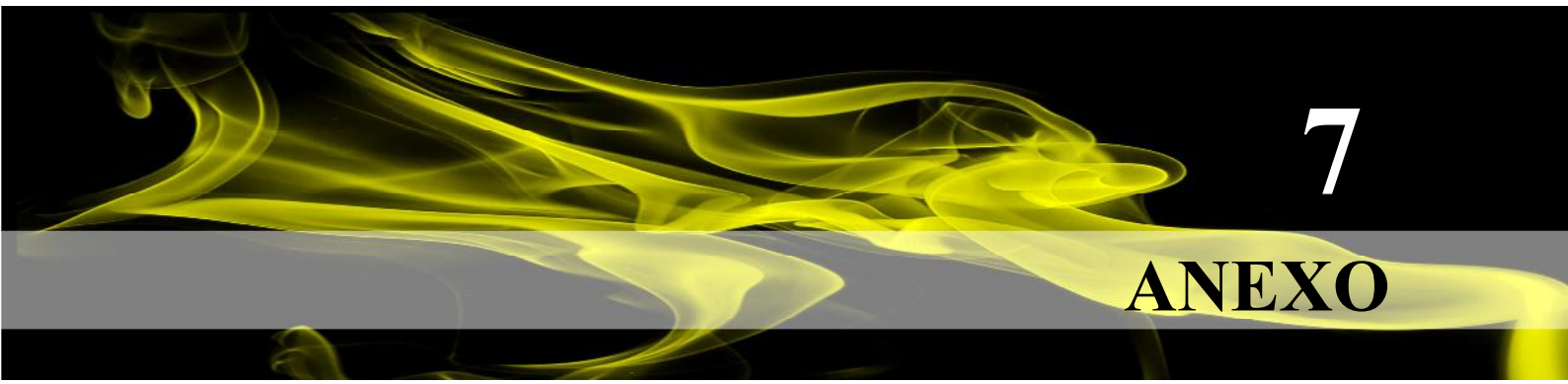
Zhang, H., Tang, B., Row, K. *Extraction of catechin compounds from green tea with a new green solvent*. Chemical Research in Chinese Universities 30 (2014) 37-41.

Zimmermann, B. F., Gleichenhagen, M. *The effect of ascorbic acid, citric acid and low pH on the extraction of green tea: How to get most out of it*. Food Chemistry 124 (2011) 1543-1548.

[a]Zosel, K. (1981). Process for the decaffeination of coffee. US 4247570

[b]Zosel, K. (1981). Process for the decaffeination of coffee. US 4260639.

Zosel, K. (1974). Process for recovering caffeine. US 3806619.



7

ANEXO

7.1. *Extraction of thymol from different varieties of thyme plants using green solvents*

were the main MH identified. Also, considerable concentrations of monoterpene alcohols (~200 mg g^{-1}) were obtained using limonene (130–180 mg g^{-1} linalool).

In the case of SFE using pure CO_2,[11] similarly to PLE and SFE/cosolvent with either ethanol or ethyl lactate, a high concentration of thymol is obtained (575 mg g^{-1}). Also, MP and their derivatives, followed by MH, were the most abundant compounds identified in SFE/CO_2 essential oil. Nevertheless, the SFE/CO_2 extracts presented higher extraction of oxygenated monoterpenes (ethers and ketones) than the rest of the extracts, with 1,8-cineole (72 mg g^{-1}) and camphor (95 mg g^{-1}) respectively being the main ether and ketone identified. The higher concentrations of these types of compounds may be attributed to the higher selectivity of CO_2 toward extraction of volatile terpene compounds.

CONCLUSIONS

The recovery of thymol from different thyme species has been reported using PLE with green solvents (ethyl lactate, ethanol and limonene) and SFE with pure CO_2 and CO_2 plus each of the three green solvents as cosolvent.

The highest recoveries were attained with *T. vulgaris* (7–11 mg thymol g^{-1} leaves), followed by *T. zygis* (6–8 mg g^{-1}) and *T. citriodorus* (~4 mg g^{-1}).

The three green solvents studied in PLE show good capacity to extract thymol from *T. vulgaris* and *T. zygis*, but no thymol could be quantified in the PLE samples of *T. citriodorus*. Furthermore, limonene was the solvent that produced the highest concentrations of thymol in the extracts owing to its lipophilic character. Although PLE proved to be a suitable technology to extract thymol from thyme plants, the highest concentrations of thymol were obtained by SFE owing to the selective extraction of lipophilic volatile compounds that could be attained with supercritical CO_2. Around 310 mg g^{-1} of thymol was obtained for *T. vulgaris* extract (15 MPa, 40 °C and CO_2/thyme ratio of 35), with a thymol recovery very similar to those obtained at 200 °C with any of the three liquid solvents in PLE. Also for *T. zygis*, concentrations were higher and recoveries were similar to PLE; in particular, SFE samples of *T. citriodorus* contain 70–80 mg g^{-1} of thymol, while very low amounts of thymol were obtained in PLE samples.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the financial support from Ministerio de Ciencia e Innovación (project 25506 FUN-C-FOOD) and Comunidad Autónoma de Madrid (ALIBIRD-CM, project S2013/ABI-2728). DVB thanks Consejo Superior de Investigaciones Científicas (CSIC) of Spain for a JAE-pre fellowship.

REFERENCES

1 Adzet T, Granger R, Passet J and San Martin R, Le polymorphisme chimique dans le genre *Thymus*: sa signification taxonomique. *Biochem Syst Ecol* **5**:269–272 (1977).
2 Pourmortazavi SM and Hajimirsadeghi SS, Supercritical fluid extraction in plant essential and volatile oil analysis. *J Chromatogr A* **1163**:2–24 (2007).
3 Prakash V, *Leafy Spices*. CRC Press, Boca Raton, FL (1990).
4 Mookherjee BD, Wilson RA, Trenkle RW, Zampino MJ and Sands KP, New dimensions in flavor research. *ACS Symp Ser* **388**:176–187 (1989).
5 Ferley J, Poutignat N, Azzopard Y and Balducci F, Aromathérapie préventive des surinfections chez les bronchiteux chroniques: evaluation statistique en milieu institutionel: contre placebo. *Phytothérapie* **24**:8 (1988).
6 Höferl M, Buchbauer G, Jirovetz L, Schmidt E, Stoyanova A, Denkova Z, *et al.*, Correlation of antimicrobial activities of various essential oils and their main aromatic volatile constituents. *J Essent Oil Res* **21**:459–463 (2009).
7 Soliman KM and Badeaa RI, Effect of oil extracted from some medicinal plants on different mycotoxigenic fungi. *Food Chem Toxicol* **40**:1669–1675 (2002).
8 Braga PC, Dal Sasso M, Culici M, Bianchi T, Bordoni L and Marabini L, Anti-inflammatory activity of thymol: inhibitory effect on the release of human neutrophil elastase. *Pharmacology* **77**:130–136 (2006).
9 Tian H and Lai DM, Analysis on the volatile oil in *Origanum vulgare*. *J Chin Med Mater* **29**:920–921 (2006).
10 Ivanovic J, Zizovic I, Ristic M, Stamenic M and Skala D, *In vitro* control of multiplication of some food-associated bacteria by thyme, rosemary and sage isolates. *Food Control* **25**:110–116 (2012).
11 García-Risco MR, Vicente G, Reglero G and Fornari T, Fractionation of thyme (*Thymus vulgaris* L.) by supercritical fluid extraction and chromatography. *J Supercrit Fluids* **55**:949–954 (2011).
12 Thompson JD, Chalchat J-C, Michet A, Linhart YB and Ehlers B, Qualitative and quantitative variation in monoterpene co-occurrence and composition in the essential oil of *Thymus vulgaris* chemotypes. *J Chem Ecol* **29**:859–880 (2003).
13 Tschiggerl F and Bucar R, Influence of saponin plants on the volatile fraction of thyme in herbal teas. *Fitoterapia* **82**:903–910 (2011).
14 Moldao-Martins M, Palavra A, Beirao da Costa ML and Bernardo-Gil MG, Supercritical CO_2 extraction of *Thymus zygis* L. subsp. *sylvestris* aroma. *J Supercrit Fluids* **18**:25–34 (2000).
15 Sacchetti G, Maietti S, Muzzoli M, Scaglianti M, Manfredini S, Radice M, *et al.*, Comparative evaluation of 11 essential oils of different origin as functional antioxidants, antiradicals and antimicrobials in foods. *Food Chem* **91**:621–632 (2005).
16 Carabias-Martínez R, Rodríguez-Gonzalo E, Revilla-Ruíz P and Hernández-Méndez J, Pressurized liquid extraction in the analysis of food and biological samples. *J Chromatogr A* **1089**:1–17 (2005).
17 Chemat F, Vian MA and Cravotto G, Green extraction of natural products: concept and principles. *Int J Mol Sci* **13**:8615–8627 (2012).
18 Gamiz-Gracia L and de Castro MDL, Continuous subcritical water extraction of medicinal plant essential oil: comparison with conventional techniques. *Talanta* **51**:1179–1185 (2000).
19 Ozel MZ, Gogus F and Lewis AC, Subcritical water extraction of essential oils from *Thymbra spicata*. *Food Chem* **82**:381–386 (2003).
20 Eller FJ and Taylor SL, Pressurized fluids for extraction of cedarwood oil from *Juniperus virginianna*. *J Agric Food Chem* **52**:2335–2338 (2004).
21 Eikani MH, Golmohammad F and Rowshanzamir S, Subcritical water extraction of essential oils from coriander seeds (*Coriandrum sativum* L.). *J Food Eng* **80**:735–740 (2007).
22 Herrero M, María C-P, Mendiola JA and Ibañez E, Compressed fluids for the extraction of bioactive compounds. *Trends Anal Chem* **43**:67–83 (2013).
23 Dawidowicz AL, Rado E, Wianowska D, Mardarowicz M and Gawdzik J, Application of PLE for the determination of essential oil components from *Thymus vulgaris* L. *Talanta* **76**:878–884 (2008).
24 Dawidowicz AL, Rado E and Wianowska D, Static and dynamic superheated water extraction of essential oil components from *Thymus vulgaris* L. *J Separ Sci* **32**:3034–3042 (2009).
25 Ivanovic J, Zizovic I, Ristic M, Stamenic M and Skala D, The analysis of simultaneous clove/oregano and clove/thyme supercritical extraction. *J Supercrit Fluids* **55**:983–991 (2011).
26 Zekovic ZP, Lepojevic Z and Vujic Dj, Supercritical extraction of thyme (*Thymus vulgaris* L.). *Chromatographia* **51**:175–179 (2000).
27 Zekovic ZP, Lepojevic ZD, Milosevic SG and Tolic AS, Modeling of the thyme–liquid carbon dioxide extraction system. *APTEFF* **34**:125–133 (2003).
28 Díaz-Maroto MC, Díaz-Maroto Hidalgo IJ, Sánchez-Palomo E and Pérez-Coello MS, Volatile components and key odorants of fennel (*Foeniculum vulgare* Mill.) and thyme (*Thymus vulgaris* L.) oil extracts obtained by simultaneous distillation–extraction and supercritical fluid extraction. *J Agric Food Chem* **53**:5385–5389 (2005).
29 Ishida BK and Chapman MH, Carotenoid extraction from plants using a novel, environmentally friendly solvent. *J Agric Food Chem* **57**:1051–1059 (2009).

30 Strati IF and Oreopoulou V, Effect of extraction parameters on the carotenoid recovery from tomato waste. *Int J Food Sci Technol* **46**: 23–29 (2011).
31 Hernández EJ, Luna P, Stateva RP, Najdanovic-Visak V, Reglero G and Fornari T, Liquid–liquid phase transition of mixtures comprising squalene, olive oil, and ethyl lactate: application to recover squalene from oil deodorizer distillates. *J Chem Eng Data* **56**:2148–2152 (2011).
32 Vicente G, Paiva A, Fornari T and Najdanovic-Visak V, Liquid–liquid equilibria for separation of tocopherol from olive oil using ethyl lactate. *Chem Eng J* **172**:879–884 (2011).
33 Golmakani M-T, Mendiola JA, Rezaei K and Ibáñez E, Expanded ethanol with CO_2 and pressurized ethyl lactate to obtain fractions enriched in γ-linolenic acid from *Arthrospira platensis* (Spirulina). *J Supercrit Fluids* **62**:109–115 (2012).
34 Villanueva Bermejo D, Luna P, Manic MS, Najdanovic-Visak V and Fornari T, Extraction of caffeine from natural matter using a bio-renewable agrochemical solvent. *Food Bioprod Process* **91**:303–309 (2013).
35 Manic MS, Villanueva D, Fornari T, Queimada AJ, Macedo EA and Najdanovic-Visak V, Solubilities of high-value compounds in ethyl lactate: measurements and modelling. *J Chem Thermodyn* **48**: 93–100 (2012).
36 Virot M, Tomao V, Ginies C, Visinoni F and Chemat F, Green procedure with a green solvent for fats and oils determination. Microwave-integrated Soxhlet using limonene followed by microwave Clevenger distillation. *J Chromatogr A* **1196/1197**:147–152 (2008).
37 Toplisek T and Gustafson R, Cleaning with D-limonene: a substitute for chlorinated solvents? *Precision Cleaning* 17–22 (1995).
38 Mamidipally PK and Liu SX, First approach on rice bran oil extraction using limonene. *Eur J Lipid Sci Technol* **106**:122–125 (2004).
39 Chemat-Djenni Z, Ferhat MA, Tomao V and Chemat F, Carotenoid extraction from tomato using a green solvent resulting from orange processing waste. *J Essent Oil Bearing Plants* **2**:139–147 (2010).
40 Fornari T, Ruiz-Rodriguez A, Vicente G, Vázquez E, García-Risco MR and Reglero G, Kinetic study of the supercritical CO_2 extraction of different plants from Lamiaceae family. *J Supercrit Fluids* **64**:1–8 (2012).
41 Baranauskiené R, Venskutonis PR, Viskelis P and Dambrauskiené E, Influence of nitrogen fertilizers on the yield and composition of thyme (*Thymus vulgaris*). *J Agric Food Chem* **51**:7751–7758 (2003).
42 Hudaib M, Speroni E, Di Pietra AM and Cavrini V, GC/MS evaluation of thyme (*Thymus vulgaris* L.) oil composition and variations during the vegetative cycle. *J Pharmaceut Biomed Anal* **129**:691–700 (2002).
43 Van den Dool H and Kratz PD, A generalization of the retention index system including linear temperature programmed gas–liquid partition chromatography. *J Chromatogr A* **11**:463–471 (1963).
44 Omidbaigi R, Sefidkon F and Hejazi M, Essential oil composition of *Thymus citriodorus* L. cultivated in Iran. *Flav Fragr J* **20**:237–238 (2005).

7.2. *Solubility of bioactive substances in ethyl lactate + water mixtures: experimental data and modeling*